ANNEXURE - IV

Relevant pages of important references cited in the patent text have been attached herewith. These references are listed below and are enclosed herewith.

(1) Title Page, Preface, and pages 64 to 65 of Natural Antioxidants: Chemistry, Health Effects, and Applications; Editor Fereidoon Shahidi; Department of Biochemistry Memorial University of Newfoundland; St. John's, Newfoundland, Canada (4 pages);

(2) Abstract of "Process for extracting antioxidants from Labiatae herbs," U.S. Patent No. 5,017,397 issued on May 21, 1991 (1 page);

(3) Abstract of "Process for manufacture of natural antioxidant products from tea and spent tea;" U.S. Patent No. 5,043,100 issued on May 21, 1999 (1 page);

(4) Abstract of "Lipid-soluble green tea catechin antioxidant solutions;" U.S. Patent No. 5,527,552 issued on June 18, 1996 (1 page);

(5) Claims 1-8 of "Lipid-soluble green tea catechin antioxidant solutions;" U.S. Patent No. 2,159,465 issued on August 25, 2000 (2 pages);

(6) Title Page, pages 464-467, 470-471, 474-475 of Bailey's Industrial Oil and Fat products, Fifth Edition, Volume 2, "Edible Oil and Fat Products, Oil and Oilseeds;" Edited by Y.H. Hui (7 pages);

(7) Pages 773-776 of "Sesaminol Glucosides in Sesame Seeds;" Hirotaka Katsuzaki, Shunico Kawakishu and Toshihuso Osawa; Department of Food Science and Technology; Nagoya University, Chikusa, Nagoya 464-01, Japan (4 pages);

ANNEXURE - IV

(8) Pages 220-226 of "Antioxidative effects of sesamol and tocopherols at various concentrations in oil during microwave heating;" Hiromi Yoshida and Sachiko Takagi (7 pages);

(9) Title Page and pages 168-168 of Principles of Food Science; Edited by Owen R. Fennema, "Part I: Food Chemistry;" Department of Food Science Co.; University of Wisconsin-Madison, Wisconsin (3 pages);

(10) Pages 1079-1086 of "Antioxidant activity of Oat Extract in Soybean and Cottonseed oils" (8 pages);

(11) Pages 281, 285-288, 291-296, 311-325 of "The Chemistry and Physiological functions of sesame," Mitsuo Namiki; Nagoya University; Chikusa-ku, Nagoya, Japan 465; Tokyo University of Agriculture; Setagaya-ku, Tokyo, Japan 156 (26 pages); and

(12) Pages 1027-1030 of "Solution of lignan Analogues to Antioxidative Activity of used unroasted Sesame Seed Oil" (4 pages)

Natural Antioxidants

Chemistry, Health Effects, and Applications

Edited by

Fereidoon Shahidi

Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland, Canada

AOCS PRESS

Champaign, Illinois

Preface

The food we consume contains many macromolecules that are susceptible to attack by free radicals. The free radical oxidation of food lipids by the chain reaction of lipid peroxidation is a major concern for both consumers and food manufacturers. Efforts are being made to control lipid oxidation in foods and to address the real biological significance to humans of dietary antioxidants and antioxidant supplements.

Oxidants are by-products of normal body metabolism and, if not controlled, may cause extensive damage to DNA, proteins, sugars, and lipids; they are contributors to aging and the degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction. Although synthetic antioxidants are generally used in foods to retain their quality, natural antioxidants such as tocopherol (vitamin E) and vitamin C as well as carotenoids act as antioxidant defenses in the body to combat diseases; antioxidant enzymes and selenium are also involved in this process.

Dietary antioxidants have generated particular interest as anticarcinogens and as defenses against the degenerative processes of aging. While most attention has been paid to small molecule dietary antioxidants such as tocopherol, ascorbate, and carotenoids, other food antioxidants may also augment the natural body antioxidants against oxidative damage and degenerative diseases. Thus, urate, albumin, bilirubin, carnosine, ubiquinol, flavonoids, and other phenolic compounds might play an important role as physiological and dietary antioxidants.

Antioxidants used in foods to control oxidation and prevent off-flavor development include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ). However, concern has been expressed about their use for this due to safety considerations. Thus, the most powerful synthetic antioxidant, namely, TBHQ is not yet allowed for food applications in Japan, Canada, and Europe, and BHA has recently been removed from the Generally Recognized As Safe (GRAS) list of compounds.

The purpose of this monograph is to provide a state-of-the-art discussion of natural antioxidants from dietary sources, their occurrence, health effects, chemistry, and methodologies. The book summarizes data on the occurrence of antioxidative compounds in cereals and legumes, oilseeds, herbs and spices, vegetables, fruits, muscle foods, and other commodities. The antioxidant vitamins and enzymes are also thoroughly discussed. The potential beneficial effects of dietary antioxidants, the chemistry of food antioxidants, and methodologies to assess lipid oxidation and antioxidant activity have also been covered. The book has been organized into chapters and sections to provide a better flow of material. The monograph would be of interest to scientists, health professionals, researchers in academia, government laboratories, and industries. It may also serve as a supplementary text book for undergraduate and graduate students in food chemistry.

Antioxidants from Spices and Herbs

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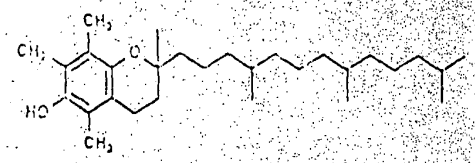
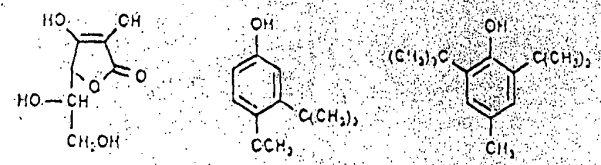
Department of Food and Nutrition, Osaka City University, Sumiyoshi, Osaka 558, Japan

Introduction

One of the principal causes of food deterioration is the oxidation of lipids. When foods are exposed to air, oxidation proceeds and results in the development of rancid odors. Deterioration by oxidation not only causes the loss of nutritive value but also produces toxic substances. To prevent the oxidative deterioration of foods, a number of efforts have been made. Among them, antioxidants have been used widely as additives to fats and foods during processing. The most common antioxidants are tocopherols, ascorbic acid, synthetic phenolic compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as shown in Fig. 4.1. These synthetic antioxidants are effective and highly stable. However, their use in food is severely restricted in several countries, because of the possibility that they may have undesirable effects on enzymes of human organs. As a result, there is a continued interest in finding safe antioxidants from natural sources. Spices and herbs are well known as excellent and widely used natural flavoring agents.



α-tocopherol



ascorbic acid BHA BHT

Fig. 4.1. Natural and synthetic antioxidants

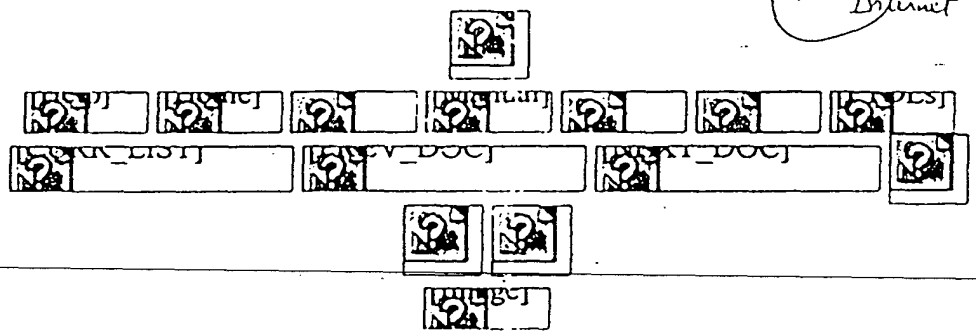
Since ancient times, spices and herbs have been used not only for flavoring foods but also for their therapeutic or medicinal properties. The preservative effect of spices and herbs suggests the presence of antioxidative and antimicrobial constituents. One of the earliest reports on antioxidants from spices appeared in 1938 in a paper by M. Tsuji (1). Some essential oils of spice such as clove oil were reported to be effective antioxidants of food oils. D. Bois and Tressler (2) discovered antioxidative activities in pepper, sage, mace, and ginger. Chipault *et al.* (3) examined [illegible] spices were [illegible] lard. [illegible] antioxidative activity in ginger and found as extracts [illegible] essential oils are less effective antioxidants in practical use. [illegible] extracts showed extremely high activity [illegible] Sen and Agarwal (5) reported the antioxidant effects of [illegible] ginger, mace, clove, and other spices on heated peanut oil. Comparative activities of spice extracts with that of BHT. A number of [illegible] properties of spices and herbs followed, including [illegible] by Lea (6), Dziezychowski (7), and Bishov *et al.* (9). Based on these [illegible] caused by rosemary, sage, and clove are remarkably active. Spices [illegible] cardamom, cassia, cinnamon, ginger, mace, [illegible] pepper, savory, thyme, and turmeric are highly effective. [illegible] antioxidative activities in the spices belonging to the family *Labiatae*, rosemary, sage, basil, marjoram, and mint. Essential oils such as [illegible] clove, allspice, cinnamon leaf, and caraway oil (Chang *et al.* (11)) [illegible] exhibited high antioxidative activity, but these compounds are limited in practical use because of their characteristic odors. The yellow pigment of turmeric, curcumin, used as a natural food colorant in curry, possesses antioxidative ability, but its practical use is also limited as is that of capsaicin, a pungent substance in red pepper. The antioxidants obtained from spices and herbs will be described in the following sections.

Antioxidants from the Labiatae (Lamiaceae)

Rosemary (Rosmarinus officinalis L.). The extract of rosemary leaves exhibited the most effective activity among the herbs. Chang *et al.* (11) reported an extraction method of the active components from rosemary and sage. Thereafter, several new techniques to prepare rosemary extract or condensate followed.

These results promoted our interest in a chemical study to isolate active antioxidants in rosemary leaves. After a combination of repeated chromatographic analyses of the weakly acidic fraction of the nonvolatile part of hexane extract, four antioxidative compounds (1–4) were isolated. The chemical structures were

AJ Internet Aug - 2000.



United States Patent 5,017,397
Nguyen , et al. May 21, 1991

Process for extracting antioxidants from Labiatae herbs

Abstract

Natural plant extracts exhibiting improved antioxidant properties are prepared from ground leaves of the Labiatae family of domestic herbs by application of a supercritical fluid extraction and fractionation process with carbon dioxide under specific operating conditions. The extracts have greater antioxidant activity than natural antioxidants extracted using other processes such as solvent extraction or molecular distillation. The extracts of the invention are oil soluble, colorless and flavorless when used at the optimum levels and provide more cost-effective protection from oxidation than existing natural antioxidants. They are effective in animal and vegetable fats and oils, processed meats and fish, processed foods and beverages, food colorants, cosmetics and health-care products at usage rates of 0.01-0.05% of fat/oil. Starting materials include Rosmarinus spp. or Salvia spp. or Thymis spp. or Origanum spp. of the common domestic herbs rosemary, sage, thyme and oregano or residues of same after removal of volatile aromatic and flavor components by means of, steam distillation, subcritical carbon dioxide or supercritical carbon dioxide at pressures of less than 350 bar.

Inventors: **Nguyen; Uy** (4635 - 37 Avenue, Edmonton, Alberta, CA); **Frakman; Grigory** (5504 - 179 Street, Edmonton, Alberta, CA); **Evans; David A.** (141 Tudor Lane, Edmonton, Alberta, CA)

Appl. No.: 514311
Filed: April 25, 1990

U.S. Class: 426/542; 426/489
Intern'l Class: A23L 001/28
Field of Search: 426/542,386,489

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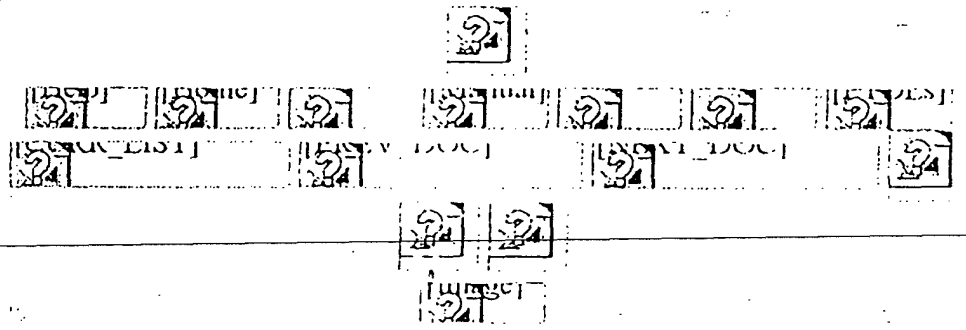
AJ Internet- Aug. 2000



United States Patent 5,043,100
Chang, et al. August 27, 1991

Process for manufacture of natural antioxidant products from tea and spent tea

Abstract

Superior oil-soluble antioxidants are produced by the vacuum steam distillation of alcohol extracts of spent black tea or spent green tea or even the tea itself.

Inventors: Chang; Stephen S. (E. Brunswick, NJ); Bao; Yongde (New Brunswick, NJ)
Assignee: Rutgers, The State University of New Jersey (New Brunswick, NJ)
Appl. No.: 481346
Filed: February 16, 1990

U.S. Class: 252/398; 426/546; 426/429
Intern'l Class: C11B 005/00; C09K 015/00
Field of Search: 426/542,429 252/398 APS

References Cited [Referenced By]

U.S. Patent Documents

3950266	Apr., 1976	Chang et al.	426/542.
4380506	Apr., 1983	Kimura et al.	252/398.
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4708820	Nov., 1987	Mamiki et al.	252/398.
4877635	Oct., 1989	Todd, Jr.	252/398.

(4)

US PATENT & TRADEMARK OFFICE

PATENT FULL TEXT AND IMAGE DATABASE

AJ June 101

United States Patent 5,527,552
Todd, Jr. June 18, 1996

ipid-soluble *green tea catechin antioxidant solutions*

Abstract

he water-soluble and fat-insoluble polyphenolic antioxidants (catechins) present in green tea are made into olution in an edible nonionic lipid-soluble solvent for the tea catechins selected from the group consisting of a tty alcohol containing 8 to 18 carbon atoms, inclusive, preferably 12 to 14 carbon atoms, inclusive, and a on-ionic surface active agent selected from the group consisting of glyceryl mono-oleate, liquid mono- and -glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid polyglycerides, olysorbate, especially glyceryl mono-oleate, and mixtures thereof, which solutions are effective antioxidants fats, oil; foods, and ingredients of foods without imparting undesirable flavors, aromas, and precipitates. ince it is known that tea polyphenols have positive effects on human health, the resulting stabilized lipids can : considered to have nutritional qualities superior to the same lipid stabilized with common synthetic ntioxidants. Unexpectedly strong synergistic effects with other natural antioxidants and with phosphates are so shown.

nventors: Todd, Jr.; Paul H. (Kalamazoo, MI)
ssignee: Kalamazoo Holdings, Inc. (Kalamazoo, MI)
ppl. No.: 352439
iled: December 9, 1994

urrent U.S. Class: 426/541; 426/545
ntern'l Class: A23B 004/00
ield of Search: 426/541,545,601,610 549/399

References Cited [Referenced By]

U.S. Patent Documents

5

AJ Intern

Patent Document Number 2159465 :
LIPID-SOLUBLE GREEN TEA CATECHIN ANTIOXIDANT SOLUTIONS

SOLUTION ANTIOXYDANTE A BASE DE CATECHINE DE THE VERT, LIPOSOLUBLE

CLAIMS:

I claim:

An antioxidant solution consisting essentially of green tea catechins dissolved in an edible non-ionic lipidsoluble solvent for the tea catechins selected from the group consisting of a fatty alcohol containing 8 to 18 carbon atoms, inclusive, and a non-ionic surface active agent selected from the group consisting of glyceryl monooleate, liquid mono- and di-glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid polyglycerides, polysorbate, and mixtures thereof.

A solution of Claim 1 wherein the edible solvent comprises a fatty alcohol containing 8 to 18 carbon atoms, inclusive.

A solution of Claim 2, wherein the fatty alcohol contains 12 to 14 carbon atoms, inclusive.

A solution of Claim 2 which is essentially free of tea lipids.

A solution of Claim 1 wherein the edible solvent comprises glyceryl mono-oleate.

A solution of Claim 5 which is essentially free of tea lipids.

A fat, oil, fatty food or food ingredient substrate stabilized-against oxidation with a solution of Claim 1.

A fat, oil, fatty food or food ingredient substrate stabilized against oxidation with a solution of Claim 1 wherein the edible solvent is a fatty alcohol containing 8 to 18 carbon atoms, inclusive.

A fat, oil, fatty food or food ingredient substrate stabilized against

d States Patent 5,132,294
ira , et al. July 21, 1992

xidative glycoside and antioxidative composition containing the

ors: Mimura; Akio (Fuji, JP); Takebayashi; Keiichi (Tsukuba,
akahara; Yoshimasa (Narashino, JP); Osawa; Toshihiko (Kasugai,

ee: Kabushiki Kaisha Kobe Seiko Sho (Kobe, JP)
No.: 724929
July 2, 1991

lass: 514/53; 514/25; 536/4.1; 536/18.1; 252/397; 426/541
Class: C09K 015/00; C07H 015/00; C07H 017/00
Search: 536/4.1 514/25,53 252/397 426/541

References Cited [Referenced By]

U.S. Patent Documents

Other References

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BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS

Fifth Edition

Volume 2

Edible Oil and Fat Products: Oils and Oilseeds

Edited by

Y. H. HUI

Technology and Commerce, International

A Wiley-Interscience Publication

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Table 10.4 Fatty acid composition of sesame oil (% of total fatty acids)

Fatty Acid	Godin and Spensley (36)	Yermanos and co-workers (32)	Seegeler (37)	Maiti and co-workers (14)
Palmitic (C16:0)	7–9	8.3–10.9	8.4–10.3	7.8–9.1
Stearic (C18:0)	4–5	3.4–6.0	4.5–5.8	3.6–4.7
Arachidic (C20:0)	8	–	0.3–0.7	0.4–1.1
Oleic (C18:1)	37–50	32.7–53.9	39.5–43.0	45.3–49.4
Linoleic (C18:2)	37–47	39.3–59.0	41.0–45.0	37.7–41.2

matter (approximately 1.0–1.2%) in sesame oil compared with those in other vegetable oils. Moreover, the unsaponifiable matter itself includes substances, such as sesamol and phytosterol, that are normally not found in other oils.

The remarkable stability of unrefined sesame oil is now widely attributed to the presence of endogenous phenolic antioxidants, viz., sesamin, sesamolin, and sesamol (or sesaminol) (Figure 10.1). Their concentrations in sesame oils and their absorption characteristics are summarized in Table 10.6. Sesamolin differs from sesamin in having an oxygen atom connecting one of its methylene

Table 10.5 Codex standards (FAO/WHO) for fatty acid composition and characteristics of sesame oil[a]

Parameter	Range
Fatty acids (%)	
C < 14	< 0.1
C14:0	< 0.5
C16:0	7.0–12.0
C16:1	< 0.5
C18:0	3.5–6.0
C18:1	35.0–50.0
C18:2	35.0–50.0
C18:3	< 1.0
C20:0	< 1.0
C20:1	< 0.5
C22:0	< 0.5
Characteristics	
Iodine value	104–120
Saponification value	187–195
Unsaponifiables (%)	2.0. max
Acid value (%)	
Virgin oil	4.0. max
Nonvirgin oil	0.6. max
Peroxide value (meq/kg)	10.0. max

[a] Source: Codex Standard 26-1981. Supplement 1, 1983.

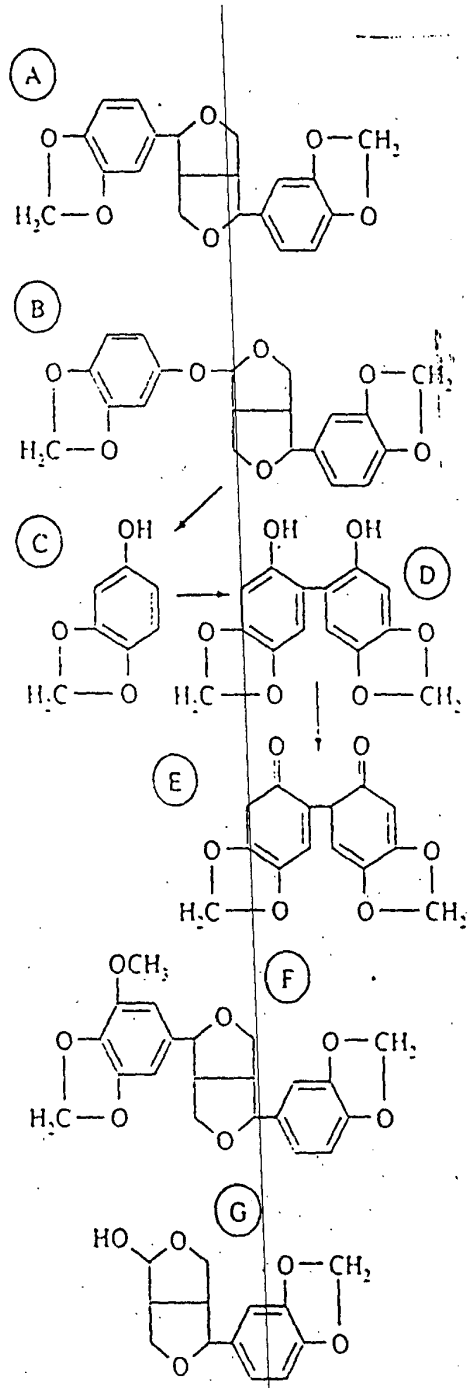

Figure 10.1 Structures of natural antioxidants found in oils from cultivated sesame. A. Sesamin; B. sesamolin; C. sesamol; D. sesamol dimer; E. sesamol dimer quinone; F. sesangolin; G. samin.

Table 10.3 Proximate composition of sesame seeds (%, moisture-free basis)[a]

Sesame Type	Fat	Protein	Carbohydrates	Crude fiber	Ash	Oxalic Acid
Sudanese white sesame						
Whole	53.3	25.0	9.5	4.1	5.4	2.7
Dehulled	57.5	29.9	5.7	3.0	3.5	0.4
Indian black sesame						
Whole	54.3	20.2	12.4	4.5	6.2	2.5
Dehulled	63.4	23.4	8.2	2.5	2.4	0.1
Husk	10.7	5.4	23.0	19.3	23.8	14.9
Dehulled (expeller-pressed)	10.6	57.8	19.4	5.4	6.6	0.3
Dehulled (hexane extracted)	0.4	60.2	27.3	5.3	6.5	0.3
Indian white sesame						
Whole	53.1	20.9	15.7	3.4	5.1	1.5
Dehulled	57.7	22.5	15.7	1.5	2.5	0.2

[a] Compiled from References 2, 3, 27 and 28.

The composition is markedly influenced by genetic and environmental factors (29). The seeds contain about 45–63% oil, averaging about 50%; 19–31% protein, averaging about 25%; 20–25% carbohydrates including the crude fiber; and 4–6% ash. In general, the Indian varieties tend to be lower in protein and higher in oil than Sudanese varieties, such as those generally appearing in the export market and are commonly used in the United States.

The hull content averages about 17% of the sesame seed, and contains large quantities of oxalic acid, calcium, other minerals, and crude fiber. Thus, when using sesame for human food, it is advisable to remove the hull. When the seed is properly dehulled, the oxalic acid content is reduced from about 3% to less than 0.25% of the seed weight (28). Screw-pressed, dehulled sesame contains about 56% protein, while the solvent extracted meal contains more than 60% protein. This is mostly used in feed except in India where it is used as a food.

7.1 Lipids

Content. Compared with many other oilseeds, sesame seeds contain more oil with a greater yield of oil on a per hectare basis. The oil content varies with genetic and environmental factors. A wide range of oil contents, from 37–63%, has been reported for sesame seed (3,6,29,30). It also varies considerably among different varieties and growing seasons (29,31). The oil content is also related to the color and size of the seed. White or light-colored seeds usually contain less oil than the dark seeds and smaller seeds more oil than larger seeds. Rough seeded cultivars generally tend to have lower oil contents than smooth seeded types (32).

Agronomic factors also influence the seed oil content. It increases with increasing length of photoperiod and early planting dates (33). Similarly, the seeds from plants with a short growing cycle tend to have higher oil contents than those from plants with a medium-to-long growing cycle. High rates of nitrogen fertilizer application reduce the oil content of sesame seeds (34).

Classification. The lipids of sesame seeds are mostly comprised of neutral triglycerides with small quantities of phosphatides (0.03 to 0.13% with lecithin:caphalin ratio of 52:46). The phosphatides also contain about 7% of a fraction soluble in hot alcohol but insoluble when cold. Sesame oil, however, has a relatively high percentage (1.2%) of unsaponifiable matter.

The glycerides are mixed type, principally oleo-dilinoleo, linoleo-dioleo triglycerides and triglycerides with one radical of a saturated fatty acid combined with one radical each of oleic and linoleic acids (29). The glycerides of sesame oil, therefore, are mostly triunsaturated (58 mol%) and diunsaturated (36 mol%) with small quantities (6 mol%) of monounsaturated glycerides. Trisaturated glycerides are practically absent in sesame oil.

The unsaponifiable matter in sesame oil includes sterols (principally comprised of β-sitosterol, campesterol, and stigmasterol), triterpenes and triterpene alcohols which include at least six compounds of which three were identified (viz., cycloartanol, 24-methylenecycloartanol, and α-amyrin), tocopherols, and sesamin and sesamolin (described below) which are not found in any other edible vegetable oils. Among the pigments spectrophotometrically identified, pheophytin A (λ_{max} 665–670 nm) was found to markedly predominate over pheophytin b (λ_{max} 655 nm) (3,29,35). The pleasant aroma and taste principles contain C_5–C_7 straight-chain aldehydes and acetylpyrazine (35).

Composition. Sesame oil is classified as a polyunsaturated, semidrying oil; it contains about 80% unsaturated fatty acids. Oleic and linoleic are the major fatty acids and are present in approximately equal amounts (Table 10.4). The saturated fatty acids, principally comprised of palmitic and stearic acids, account for less than 20% of the total fatty acids. Arachidic and linolenic acids are present in very small quantities (38). Heptadecanoic acid (0.2–0.3%) and hexadecenoic acid (0.0–0.5%) have also been reported from some sesame seed oils (3,35).

The ranges of fatty acid composition tentatively adopted by the Food and Agriculture Organization and the World Health Organization Codex Alimentarius Committee on Fats and Oils for sesame oil are summarized in Table 10.5. Codex Standards also advise values for some of the characteristics of sesame oil (see Table 10.5).

Endogenous Antioxidants. Among the commonly used vegetable oils, sesame oil is known to be most resistant to oxidative rancidity (39). It also exhibits noticeably greater resistance to autoxidation than would be expected from its content of tocopherols (vitamin E). This unusual stability to oxidat[illegible] is often attributed to the presence of a large proportion of unsa[illegible]

slightly higher activity. Kikugawa and co-workers (45) noted that the sesamol dimer, an oxidation product of sesamol, has an extensive antioxidant activity which in many cases was higher than that of sesamol in some oils.

Kikugawa and co-workers (45) also evaluated the relevance of sesamolin and sesamol to the stability of sesame oil using a similar approach as above (Figure 10.3). Edible sesame oil containing both sesamolin and sesamol resisted autoxidation; the POV did not increase and was less than 5 meq/kg even after 94 h of activating the oil (Figure 10.3*A*). In contrast, the Japan Pharmacopoeia (JP) sesame oil containing sesamolin but free from sesamol was relatively unstable. Its POV rapidly increased to about 100 meq/kg after 23 h of activating without exhibiting a clear induction period. When 0.01% sesamol was incorporated in JP sesame oil, its stability increased slightly, but still was significantly lower than that of the edible sesame oil. Addition of 0.01% sesamol dimer, however, did increase the stability of JP oil to oxidation.

When JP oil was mixed with 5% edible oil (total sesamol content of 0.0005%), its stability was found to be similar to that of JP oil containing 0.01% sesamol. Addition of 10% edible oil (0.001% total sesamol content) further improved its stability to oxidation (Figure 10.3*B*).

The results of Kikugawa and co-workers (45) therefore, suggest that edible sesame oil contains antioxidative compound(s) other than sesamol which give its highly stable character. Moreover, bound sesamol (sesamolin) is inactive as an antioxidant in various fat-based systems. In their study, only sesamol dimer, a possible intermediate of the oxidative degradation of sesamol, showed a potent antioxidant activity. Its structure was also similar to that of the dimeric compounds of butylated hydroxyanisole (BHA) that are commonly produced in activated oils containing BHA. Moreover, sesamol content in edible unrefined sesame oil increases with storage. The presence of sesamol, therefore, appears to be a mere index of whether the oil is protected against autoxidation by another antioxidant(s).

The long established Villavecchia color test or its modification, the Baudouin test, gives a cherry red coloration with strong hydrochloric acid and furfural or sucrose in the presence of sesamolin or free sesamol. Hence, the addition of sesame oil to margarine and vanaspati (a type of shortening common in India) was a legal requirement in several countries to detect their use as adulterants in butter or ghee made from cow's milk (41). Moreover, the use of sesame oil along with hydrogenated fats in products, such as vanaspati, also brings additional desirable linoleate to their formulations. The compulsory use of these tests, however, is decreasing in favor of IR, UV, and chromatography tests.

It should also be noted that frequently the milk of animals fed with sesame oil cake or meal gives a positive test for sesame oil (46). Sometimes, due to its popularity and higher price, sesame oil is adulterated with less expensive oils. The more common oils used for this purpose are rape (or canola), poppy seed, cottonseed, and groundnut oils. Rapeseed oil lowers the saponification value; poppy seed oil raises the iodine number; cottonseed oil, besides increasing the titer of the fatty acids, usually responds to the Halphen test; and groundnut oil can be detected by separating an arachidic and lignoceric acid mixture.

The addition of sesame oil to other fat-based systems may also enhance the stability of vitamin A. In one such study reviewed by Weiss (3), the stabilizing action of various oils after storage in the dark for five months at 4°C indicated that the amount of β-carotene which disappeared was 8%, refined cottonseed oil; 15%, sesame oil; 25%, coconut oil; 38%, olive oil; 45%, corn oil; and 62%, crude wheat germ oil. This protective action on β-carotene compared with most other vegetable oils is probably related to the antioxidant activity of sesamol and other related derivatives of sesame oil.

Among the commercial antioxidants, sesamol has been reported second only to propyl gallate and nordihydroguairetic acid for stabilizing lard (6,44). Sesamin has enjoyed important use as an adjuvant to pyrethrum insecticides because of its synergistic effect on the latter and consequent lowering of cost (2,3,44). Synthetic sesamol for antioxidant and insecticidal use is commercially available.

Properties. A number of parameters are used to characterize vegetable oils. Data on some important characteristics of sesame oil are summarized in Table 10.8. Sesame oil is dextrorotatory, which is unusual for an oil devoid of optically active fatty acid glycerides (3). The unsaponifiable fraction of the oil, however, does contain optically active minor constituents, which are responsible for the optical rotation of the oil.

Table 10.8 Characteristics of sesame oil

Parameter	Andraos and co-workers (47)	Lyon (29)	Seegeler (37)	Weiss (6)
Specific gravity (25°/25°C)	0.918	0.918–0.921	0.916–0.921	0.922–0.924
Refractive index (n_D^{25})	1.463 (25°C)	1.472–1.474 (25°C)	1.463–1.474 (25°C)	1.458 (60°C)
Smoke point (°C)	165	–	166	–
Flash point (°C)	339	–	375	–
Solidifying point (°C)	–	–	−3 to −4	−3 to −4
Titer (°C)	22	20–25	20–25	22–24
Free fatty acids (as % oleic)	1.0	–	1.0–3.0	1.0–3.0
Unsaponifiable matter (%)	2.3	1.8	0.9–2.3	0.9–2.3
Iodine value	112	104–118	103–130	103–116
Saponification value	186	187–193	186–199	188–193
Reichert-Meissel value	0.51	–	0.1–0.2	0.1–1.0
Polenske value	0.47	–	0.10–0.50	–
Hydroxyl number	5.3	–	1.0–10.0	1.0–10.0
Thiocyanogen value	76	–	74–76	74–76
Hehner value	–	–	96.0	95.7

Table 10.6 Concentrations of sesamin, sesamolin, and sesamol in sesame oils and their absorption characteristics (40)

Parameter	Sesamin	Sesamolin	Sesamol
Concentration (mg/100 g oil)	293–885	123–459	Trace–5.6
λ_{max}	287	288.5	296
ε_{max}	23.0	21.8	29.7
λ_{max}	236	235	233
ε_{max}	26.0	24.9	21.2

dioxyphenyl groups to the central tetrahydrofurfurofuran nucleus. Sesame oil has a characteristic UV absorption with two maxima, at 287.5 and 235 nm. This absorption of sesame oils is, most likely primarily if not exclusively, due to its content of sesamin, sesamolin, and sesamol (41).

Presumably, the superior oxidation stability of sesame oil is due to sesamol. It is a very minor component of the oil, and only small quantities of the free substance are found in the natural oil. Sesamol, however, is capable of generating from sesamolin by intermolecular transformation during the industrial bleaching process of unroasted sesame oil with acid-type bleaching earths, by dilute mineral acids, by hydrogenation, or during the frying process (2,6,42). Alkali neutralization, washing, and deodorization diminish released sesamol. After the action of dilute acids has released sesamol from sesamolin, the remaining compound is samin (Figure 10.1). In contrast, sesamin gives episesamin during the bleaching process. Refined deodorized sesame oil usually contains only traces of free sesamol and is, thus, no more stable than other similar unsaturated oils.

Recently, Tashiro and co-workers (42) have investigated the minor components of 42 dehiscent types of cultivated sesame from China, Colombia, Mexico, Afghanistan, and Vietnam. These strains provided a wide range of types that differed in height, degree of branching, maturity, and other agronomic characteristics. These strains included 15 white-, 12 brown-, 11 black-, and 2 yellow-colored seed types. The results from this study are summarized in Table 10.7.

The sesamin content of the oils ranged from 0.07 to 0.61%, averaging 0.36%, whereas the sesamolin content ranged from 0.02 to 0.48%, averaging 0.27%. The highest sesamin value found in this study was for a sample of a white-seeded strain having a high oil content of 55%, whereas the lowest value was for a black-seeded strain having a low oil content of 44.6%. In contrast, the highest and the lowest values for sesamolin were found in samples from the white-seeded strains.

Tashiro and co-workers (42) further investigated the effects of seed color on both the oil content and the minor components in the oil. The black seed types in this study contained significantly less oil (Table 10.7). These differences were also seen in the sesamin content with black-seeded strains

Table 10.7 Sesamin and sesamolin contents in oils from cultivated sesame (42)[a]

Seed type	Oil (%)	Sesamin (%)	Sesamolin (%)
42 Strains (overall)	52.7 (43.4–58.8)	0.36 (0.07–0.61)	0.27 (0.02–0.48)
White-seeded (15 strains)	55.0 (51.8–58.8)	0.44 (0.12–0.61)	0.25 (0.02–0.48)
Brown-seeded (12 strains)	54.2 (50.5–56.5)	0.36 (0.11–0.61)	0.30 (0.13–0.42)
Black-seeded (11 strains)	47.8 (43.4–51.1)	0.24 (0.07–0.40)	0.27 (0.13–0.40)

[a] Mean values with ranges in parentheses.

averaging almost 50 and 25% lower sesamin content, respectively, compared with those found in white- and brown-seeded strains. In contrast, the data for sesamolin showed no consistent differences among the various color types. In this study, the oil and sesamin contents were significantly and positively correlated in both the white- and black-seeded strains.

Among other species related to *S. indicum*, oils from *S. angustifolium* contain both sesamin and sesamolin, *S. radiatum* only sesamin, while *S. alatum* oils are devoid of both compounds (43). Sesamolin has not been found to occur in any genera other than *Sesamum*, although sesangolin has been found in the oil of *S. angolense* (3,44). In contrast, sesamin has been isolated from the bark of various *Fagara* species, *Flindersia pubescens*, *Chamaecyparis obtusa*, and *Ocotea usambarensis*; from the heartwood of *Gingko biloba* and from *Evodia micrococca* var. *pubescens*; and from the fruit of *Piper guineense* (3).

The comparative antioxidant activities of sesamol and related compounds in various fats and oils have been evaluated by several researchers. An extensive investigation in this regard was carried out by Kikugawa and co-workers (45). These researchers separated sesamolin, sesamol, and its possible oxidation product, a sesamol dimer (Figure 10.1) in sesame oil by high performance liquid chromatography (HPLC), and elucidated the relevance of these constituents to the stability of sesame oil as well as other fat and oil-based systems.

In one experimental system, Kikugawa and co-workers (45) added sesamolin, sesamol, and sesamol dimer to lard, methyl oleate, and soybean oil at a concentration of 0.01% and evaluated the stability of these fats by monitoring the peroxide value (POV) using the active oxygen method at regular intervals. Sesamolin showed no significant antioxidant activity in any of the fats or oils (Figure 10.2). In contrast, sesamol and sesamol dimer exhibited extensive antioxidant activity as indicated by the prolonged induction periods, especially in lard and methyl oleate. While the activity of sesamol dimer was higher than that of sesamol in lard, it was lower in methyl oleate. In soybean oil, sesamol exhibited low but significant activity, while the sesamol dimer had

Pergamon

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SESAMINOL GLUCOSIDES IN SESAME SEEDS

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Key Word Index—*Sesamum indicum*; Pedaliaceae; seeds; lignan glucosides; sesaminol; antioxidants.

Abstract—The structures of novel sesaminol glucosides isolated from sesame seed were determined to be sesaminol 2′-*O*-*β*-D-glucopyranoside, sesaminol 2′-*O*-*β*-D-glucopyranosyl (1→2)-*O*-*β*-D-glucopyranoside and sesaminol 2′-*O*-*β*-D-glucopyranosyl (1→2)-*O*-[*β*-D-glucopyransyl (1→6)]-*β*-D-glucopyranoside.

INTRODUCTION

We have been involved in the isolation and structural determination of lignan and flavonoid glucosides in plant materials, in particular, sesame seed [1, 2] and young green barley leaves [3]. These lignan and flavonoid glucosides show strong antioxidative activity both in chemical and biological model systems. Now we have succeeded in isolating precursors of antioxidants which were determined to be novel sesaminol mono-, di- and triglucosides. These compounds, especially, mono- and diglucosides were resistant to hydrolysis by *β*-glucosidase. In this paper, we report the structural determination of these compounds and discuss their hydrolytic behaviour.

RESULTS AND DISCUSSION

Sesame seed (250 g) was ground and defatted with *n*-hexane and extracted with 80% ethanol. The 80% ethanol extract was dissolved in 50 mM acetate buffer pH 5.4 and hydrolysed overnight with *β*-glucosidase. The reaction mixture was extracted with ethyl acetate and the extract purified by preparative HPLC to give six compounds. Compounds 1–4 were identified as pinoresinol (1) [4], PI (5), sesamolinol (3) [6] and sesaminol (4) [7] by comparison of analytical data with those of authentic samples. Compound 7 was isolated from the 80% ethanol extract of sesame seed using an XAD-2 column and preparative HPLC (ODS).

Compound 5 showed a $[M+Na]^+$ peak at *m/z* 555 in positive FAB-mass spectrometry. The ^1H NMR (500 MHz) showed the presence of methylene dioxy groups (*δ* 5.93 and 5.96) and a furanofuran moiety (*δ* 2.98, 3.06, 4.19, 4.26, 4.63 and 5.18). The anomeric region showed a 1,3,4-trisubstituted ring (*δ* 6.78, 6.81, and 6.91) and a *para*-substituted ring (*δ* 6.82, 2H, *s*). The chemical shifts were identical to those of sesaminol, suggesting that 5 had sesaminol as its aglycone. The other chemical shift indicates that 5 had a D-glucose moiety. Coupling constants of the anomeric proton of D-glucose at *δ* 4.85 (7 Hz) indicates that the anomeric configuration was *β* [8]. Moreover, ^{13}C NMR spectra also confirmed that 5 had a sesaminol moiety and one molecule of glucose in its structure (Tables 1 and 2). Compound 5 generated sesaminol and methyl glucoside by methanolysis. Sesaminol was identified by comparison of its retention time by HPLC with an authentic sample. The GC retention time of the TMSi derivative of the sugar part was exactly the same as that of an authentic TMSi derivative of methyl glucoside. Since the HMBC spectrum showed a cross-peak at C-2′/H-G1, sesaminol had glucose at the 2′-position. From these results, 5 was determined to be sesaminol 2′-*O*-*β*-D-glucopyranoside.

Compound 6 also has the same chemical shift for the aglycone moiety as sesaminol but contained two sugars in its structure. The positive FAB mass spectrum showed a $[M+Na]^+$ peak at *m/z* 717. Methanolysis yielded sesaminol and D-glucose as products. The aglycone was identified as sesaminol by RP-HPLC. The sugar was characterized as glucose by GC of its TMSi derivatives. The mass spectral and methanolysis data indicated that 6 was a sesaminol diglucoside. The linkage pattern and anomeric configuration of the sugar moiety were determined by NMR spectroscopy, including HMBC. ^1H and ^{13}C NMR chemical shift data are given in Tables 1 and 2. In the ^1H NMR spectrum of 6, the two doublets at *δ* 4.63 and 4.96, were ascribed to the anomeric protons. The anomeric configurations were deduced from the coupling constants. Values obtained for the coupling constants of the anomeric proton of D-glucose (7 and 8 Hz) were characteristic of the *β*-configuration. The individual chemical shifts of the sugar moiety were readily identified from the 2D NMR spectra (^1H–^1H COSY, HSQC and HMBC). The coupling constants of the sugar moiety were second order but not identified; chemical shifts were

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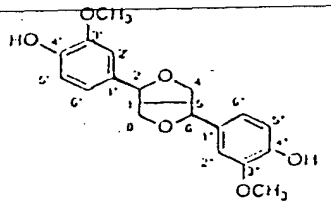
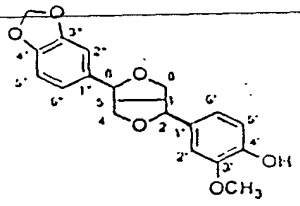
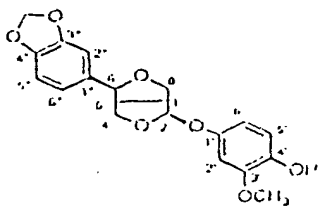


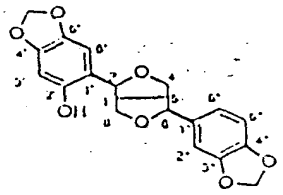
Compound 1 (Pinoresinol)



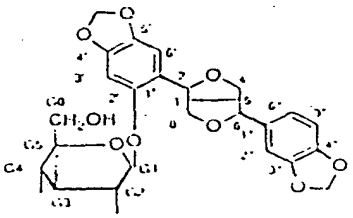
Compound 2 (P1)



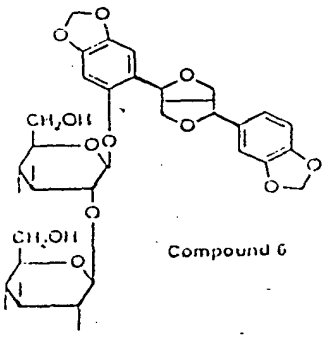
Compound 3 (Sesamolinol)



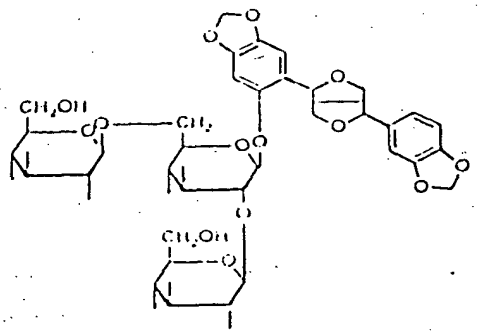
Compopund 4 (Sesaminol)



Compound 5



Compound 6



Compound 7

assigned by a HSQC spectrum. The sugar sequence and point of attachment to the aglycone were evident from the HMBC spectrum; in which long-range couplings between C-2'/H-G1 and C-G2/H-G1' were observed. The chemical shift of C-G2 at δ81.4 showed a downfield shift in comparison with C-G2' at δ74.9. From these results, the structure of **6** was determined to be sesaminol 2'-*O*-*β*-D-glucopyranosyl (1→2)-*O*-*β*-D-glucopyranoside.

Table 1. ^{1}H NMR spectral data of 5–7 (270 MHz)

	Compound		
	5	6	7
	2.98 *m*	2.81 *m*	2.88 *m*
	5.18 *d* 4	5.06 *d* 5	4.65 *d* 3
	4.06 *dd* 9, 4	3.78 *dd* 9, 4	3.76*
	4.26 *dd* 9, 6	4.18 *dd* 9, 4	4.16 *m*
	2.98 *m*	2.89 *m*	2.88 *m*
	4.63 *d* 5	4.59 *d* 6	5.16 *d* 3
	3.87 *dd* 9, 4	4.00 *dd* 9, 4	3.98 *dd* 8, 3
	4.19 *dd* 9, 6	4.18 *dd* 9, 4	4.16 *m*
	6.82 *s*	6.80 *s*	6.91 *s*
	6.82 *s*	6.82 *s*	6.75*
	6.85 *d* 2	6.91 *d* 2	6.75*
	6.78 *d* 8	6.84 *d* 7	6.77*
	6.81 *dd* 8, 2	6.83 *dd* 7, 2	6.75*
CH_2-O-	5.93 *s*	5.95 *s*	5.87 *s*
CH_2-O-	5.96 *s*	5.99 *s*	5.91 *s*
	4.85 *d* 7	4.96 *d* 7	5.19 *d* 7
	3.50	3.56	3.84
	3.50	3.20	3.81
	3.50	3.21	3.50
	3.50	3.54	3.72
	3.72	3.55	3.80
	3.94	3.70	4.09
		4.63 *d* 8	4.85 *d* 8
		3.03	3.29
		3.10	3.48
		3.19	3.40
		3.30	3.30
		3.41	3.58
		3.53	3.65
			4.39 *d* 8
			3.21
			3.39
			3.29
			3.17
			3.60
			3.62

*Coupling constants for 2–6 protons of sugar moieties and * in second order.

†Chemical shifts were assigned by HSQC spectra.

Table 2. ^{13}C NMR spectral data of 5–7 (67.5 MHz)

	Compound		
C	5	6	7
1	55.4	54.2	55.4
2	82.1	80.5	83.0
4	71.9	71.0	73.5
5	54.7	53.9	55.4
6	85.9	84.4	87.4
8	73.6	72.3	74.5
1′	125.5	124.0	125.4
2′	150.4	148.1	150.0
3′	99.6	97.5	100.4
4′	147.8	146.5	149.4
5′	143.3	141.5	144.8
6′	105.8	104.6	107.5
1″	136.8	135.5	136.3
2″	107.2	106.5	108.8
3″	148.7	147.3	149.6
4″	147.1	146.3	149.0
5″	108.6	107.9	110.5
6″	120.1	119.3	122.0
-O-CH_2-O-	102.0	101.0	103.4
-O-CH_2-O-	101.9	100.9	103.4
G1	103.4	98.7	101.1
G2	74.7	81.4	82.4
G3	77.8	76.8	77.7
G4	71.2	69.5	71.4
G5	78.0	76.6	77.4
G6	62.6	60.3	70.5
G1′	—	104.0	103.9
G2′	—	74.9	76.0
G3′	—	77.0	77.7
G4′	—	69.3	71.4
G5′	—	76.3	78.0
G6′	—	60.6	63.0
G1″	—	—	104.7
G2″	—	—	75.3
G3″	—	—	77.9
G4″	—	—	71.8
G5″	—	—	78.1
G6″	—	—	62.4

Compound 7 was a precursor of 5 and 6. The negative FAB-mass spectrum showed a $[M-H]^-$ peak at *m/z* 855. Hydrolysis of 7 gave sesaminol and D-glucose as products. Compound 7 was thus comprised of sesaminol and three D-glucoses. The anomeric configuration of 7 was also determined to be *β* on the basis of the coupling constants of the anomeric proton of 7 at *δ* 4.39 (8 Hz), 4.85 (7 Hz) and 5.19 (7 Hz). One of the 2-position carbon signals of the D-glucosyl residue at *δ* 82.4 showed a downfield shift in comparison with the other 2-position carbon signal of the D-glucosyl residues observed at *δ* 75.3 and 76.0. The same downfield shift was observed in the signal of one of the 6-position carbon signals of the D-glucosyl residue (*δ* 70.5, 63.0 and 62.4). The 1D TOCSY and HSQC spectra showed downfield shifted protons in the same D-glucosyl residue. The sugar sequence was determined to be branched (1→2) and (1→6) linked. From these results, 7 was determined to be sesaminol 2′-*O*-*β*-D-glucopyranosyl (1→2)-*O*-[*β*-D-glucopyranosyl (1→6)]-*β*-D-glucopyranoside.

Compounds 5 and 6 were resistant for hydrolysis by *β*-glucosidase. These compounds are assumed to be resistant because of steric hindrance. It is thought that *β*-glucosidase cannot attack the sugar moieties of mono- and diglucosides because they are very close to an aromatic ring of sesaminol, although the detailed reaction mechanisms are still unknown.

Our data suggest that sesame seeds contain both antioxidative and non-antioxidative lignan glucosides in large quantities. Antioxidative lignan glucosides, e.g. pinoresinol glucosides, KP1, KP2 and KP3, may play an important role in antioxidative defence systems against

oxidative damage caused during the storage of sesame seeds. On the other hand, newly isolated non-antioxidative lignan glucosides and sesaminol glucosides have no role in the antioxidative defence systems, although these sesaminol glucosides can be hydrolysed to form sesamol, a strong antioxidative lipid-soluble lignan, by intestinal β-glucosidase after ingestion of sesame seeds [9]. Sesaminol has already been reported to show strong antioxidative activity both in *in vitro* and *in vivo* systems [10], and to show synergistic effects in raising liver and plasma concentrations of vitamin E in rats [11].

EXPERIMENTAL

General. ^{1}H NMR (270 MHz) and ^{13}C NMR (67 MHz). EI-MS direct insertion probe at 70 eV. FAB-MS: Xe gas and glycerin as matrix. Column: Amberlite XAD-2. HPLC column: Develosil ODS-5 or 10 and Develosil SI-60-5 (Nomura Chemistry), detector UV at 285 nm.

Plant material. Sesame seed (*Sesamum indicum* L.).

Extraction and isolation. Sesame seed (80 g) was defatted with *n*-hexane and extracted with 80% EtOH (2 l × 3). The extract (4.8 g) was incubated at 37° for 8 hr with β-glucosidase (5 unit ml^{-1}) in 50 mM acetate buffer (pH 5). The reaction mixt. was extracted with EtOAc. The EtOAc extract was fractionated into S1–S6 using prep. HPLC under the following conditions: column Develosil ODS-10 (250 × 20 mm i.d.), solvent MeOH–H_2O (3:2), flow rate 6 ml min^{-1}. S1 was purified to 1 by prep. HPLC under the following conditions: column Develosil ODS-5 (250 × 10 mm i.d.), solvent MeOH–H_2O (2:3), flow rate 2.5 ml min^{-1}. S2 was purified to 6 by prep. HPLC under the following conditions: column Develosil ODS-5 (250 × 10 mm i.d.), solvent MeOH–H_2O (1:1), flow rate, 2.5 ml min^{-1}. S3 was purified to 5 by prep. HPLC under the following conditions: column Develosil ODS-5 (250 × 10 mm i.d.), solvent, MeOH–H_2O (1:1), flow rate 2.5 ml min^{-1}. S4 was purified by prep. HPLC under the following conditions: column Develosil pH 7 (250 × 8 mm i.d.), solvent MeOH–H_2O (3:2), flow rate 3 ml min^{-1}, and finally purified to 2 under the following conditions: column Develosil SI-60-5 (250 × 8 mm i.d.), solvent, *n*-hexane–EtOAc (1:1), flow rate 3 ml min^{-1}. S5 was purified to 3 by prep. HPLC under the following conditions: column Develosil ODS-5 (250 × 10 mm i.d.), solvent MeOH–H_2O (3:2), flow rate 2.5 ml min^{-1}. S6 was purified to 4 by prep. HPLC under the following conditions: Develosil ODS-5 (250 × 10 mm i.d.), solvent MeOH–H_2O (3:2), flow rate 2.5 ml min^{-1}. Finally, 13 mg 1, 6.9 mg 2, 12.1 mg 3, 6.7 mg 4, 8.9 mg 5 and 113.8 mg 6 were obtained.

The 80% EtOH extract (0.5 g) was charged into an Amberlite XAD-2 column and eluted with H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, MeOH and Me_2CO. The 60% MeOH fr. (74.8 mg) was then purified by prep. HPLC under the following conditions: column, Develosil ODS-5 (250 × 10 mm i.d.), solvent MeOH–H_2O (1:1), flow rate 2.5 ml min^{-1}. Pure 7 (19.0 mg) was obtained.

Methanolysis. Methanolysis was carried out in 5% HCl–MeOH at 80° for 2 hr.

Analysis of methanolysis products. The EtOAc extracts of methanolysis products were analysed under the following conditions: column Develosil ODS-5 (150 × 4.6 mm i.d.), solvent MeOH–H_2O (3:2), flow rate 1 ml min^{-1}. The TMSi derivatives of the H_2O layer of the methanolysis products were analysed by GC under the following conditions: column 3% silicone GE SE-52 (2 m × 4 mm i.d.), column temp. 180°, carrier gas N_2 at 40 ml min^{-1}, detector FID.

Compound 5. $[\alpha]_D^{23}$ −6.24 (MeOH; c 1.3). UV λ_{max}^{MeOH} nm: (log ε): 290 (3.82), 236 (3.80). IR ν_{max}^{KBr} cm^{-1}: 3410, 1630, 1510, 1250, 1040. FAB-MS (pos.) *m/z* 555 [M + Na]$^+$. ^{1}H NMR (acetone-d_6): see Table 1. ^{13}C NMR: see Table 2.

Compound 6. $[\alpha]_D^{23}$ −25.3 (MeOH; c 0.2). UV λ_{max}^{MeOH} nm: (log ε): 290 (3.94), 236 (4.0). IR ν_{max}^{KBr} cm^{-1}: 3390, 1630, 1490, 1250, 1090, 1040. FAB-MS (pos.) *m/z* 717 [M + Na]$^+$. ^{1}H NMR (DMSO-d_6): see Table 1. ^{13}C NMR: see Table 2.

Compound 7. $[\alpha]_D^{23}$ −9.75 (H_2O; c 0.3). UV $\lambda_{max}^{H_2O}$ nm: (log ε): 288 (3.88), 235 (3.97). IR ν_{max}^{KBr} cm^{-1}: 3410, 1630, 1510, 1250, 1040. FAB-MS (neg.) *m/z* 855 [M−H]$^+$. ^{1}H NMR (D_2O): see Table 1. ^{13}C NMR: see Table 2.

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Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating

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Abstract: The effectiveness of sesamol and tocopherols or their mixtures at different concentrations (50 to 800 ppm) on the oxidative stability of tocopherol-stripped oils was studied under microwave heating conditions. Microwave heating accelerated the oxidation of the purified substrate oils. The oxidative deterioration of the oils was significantly ($P < 0.05$) retarded during microwave heating by the addition of sesamol or tocopherols, and also mixtures of these antioxidants. A combination of sesamol and γ-tocopherol was more efficient than that of sesamol and the other tocopherol homologues in inhibiting hydroperoxide formation in the oils. Useful levels of these antioxidants were 400 ppm for tocopherols and 50–400 ppm for sesamol. In general, the residual amount of sesamol in the oils during microwave heating was significantly greater ($P < 0.05$) than that of tocopherols. Very effective combinations of tocopherols and sesamol as antioxidants for the purified oils were 200 or 400 ppm of γ-tocopherol and 50, 200 or 400 ppm of sesamol, respectively.

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Keywords: anisidine value; antioxidants; carbonyl value; microwave heating; peroxide value; purified vegetable oils; sesamol; tocopherol homologues

INTRODUCTION

Recently, as the development of processed foods containing fats and oils has increased, the rancidity caused by lipid oxidation poses a serious problem. Controlling oxidation in natural and processed foods is a difficult aspect of food preservation, even in low-fat foods.[1] Lipid oxidation not only produces characteristic undesirable odours and flavours, but also decreases the nutritional quality and safety of foods by the formation of secondary reaction products during cooking and processing.[2,3] The addition of antioxidants to fat- and oil-based products is one of the most efficient methods to prevent oxidation of the lipids. There are some questions regarding safety of synthetic compounds,[4] so research efforts have focused on natural antioxidants in biological and food systems.[5] Sesamol has been generally regarded as the main antioxidative component in sesame seeds.[6–8] However, Yoshida *et al*[9] reported that commercial raw sesame seeds contain only trace amounts of sesamol, and a significant level of tocopherol, mainly γ-tocopherol, which nevertheless cannot account completely for the stability of crude sesamol oil. Sesame oil is characterised by the presence of a number of compounds from the furofuran family, mainly sesamin and sesamolin.[7] Sesamol can be liberated from sesamolin during seed roasting,[10] frying[11] and hydrogenation.[12]

Tocopherols, in addition to possessing vitamin E function,[13] are the major natural antioxidants in foods and are important for the stability of vegetable oils. Tocopherol chemistry has been studied extensively[14–18] especially with regard to the relative antioxidant activities of α-, γ-, and δ-tocopherols, the forms commonly found in vegetable oils. Tocopherols are not volatile, as are butylated hydroxytoluene and butylated hydroxyanisole, and they do not cause off-flavour, as does tertiary butylhydroquinone, or discoloration, as does lecithin at higher temperatures;[19] therefore, they can be used for stabilising heated oils. Yoshida *et al*[9] reported on the antioxidant activities of individual tocopherols in different lipid systems during microwave heating.

The aim of this work was to study the antioxidative properties of tocopherols and sesamol or their mixtures at various concentrations on the oxidative stability of purified (tocopherol-stripped) substrate oils when heated in a microwave oven.

MATERIALS AND METHODS

Vegetable oils

Rapeseed, soya bean and safflower oils, with different degrees of unsaturation (by iodine values (IV)), were used as the substrates. Rapeseed (IV = 108.5), soya bean (IV = 132.0) and safflower (IV = 148.0)

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oils were purchased from Nacalai Tesque Inc (Kyoto, Japan). The oils had been degummed, bleached, alkali-refined and deodorised, by the manufacturer's company, and were free of added antioxidants and preservatives. Tocopherol-stripped oils were prepared from these oils by aluminium oxide column chromatography,[19] immediately prior to use. The aluminium oxide was washed with deionised water and activated at 200 °C for 10 h before use. Chlorophylls, phospholipids and free fatty acids were determined for the same oil sample and its purification according to the methods Ca 7a-40, Ja 19-86 and Ca 9a-40,[19] respectively. Tocopherols in commercial and tocopherol-stripped oils were determined by normal-phase high-performance liquid chromatography (HPLC) as described below. Fatty acid methyl esters were prepared[20] from tocopherol-stripped oils and their compositions were analysed by a Shimadzu Model 14A gas chromatograph (GC) as described previously.[21]

Antioxidants

Sesamol (reagent grade, 98.0 g kg^{-1}) was purchased from Aldrich Chemical Co (Milwaukee, WI, USA). Vitamin E homologues (α, γ and δ) were purchased from Eisai Co (Tokyo, Japan) and were of the *d*-form (*RRR*-). The purity of each tocopherol was 985 g kg^{-1} as determined by HPLC. Each antioxidant was added directly to the tocopherol-stripped oils as a *n*-hexane solution for tocopherols or a benzene solution for sesamol. The mixtures were stirred at 25 °C for 30 min to ensure complete dissolution of the antioxidants in the oils. The solvent was removed by evaporation under a stream of nitrogen before microwave heating. A control sample with no added antioxidants was prepared under the same conditions described above.

Microwave heating treatment

Purified substrate oils containing various amounts of tocopherols and sesamol or their mixtures (50, 100, 200, 400 or 800 ppm) were separately prepared. Samples (5.0 g) were divided into a 25 ml brown bottle and sealed with polyethylene film. All oil samples were prepared in replicate and then simultaneously heated at a frequency of 2450 MHz for each time period in a microwave oven, as reported previously.[21] Treatment time varied from 1 to 25 min, at intervals of 1 or 3 min. The temperature of the oils was immediately taken after each microwave treatment as described previously.[21] A control sample was prepared for each exposure time with the individual tocopherol-stripped oils.

Chemical characteristics of substrate oils

After fixed time intervals, the carbonyl values and *p*-anisidine values of the heated oils were determined by JOCS methods[22] and IUPAC methods,[23] respectively. Peroxide values and IVs were measured as AOAC methods 28.022 and 28.020, respectively.[24]

Analysis of antioxidants

A 2 g portion of each oil sample, before and after microwave heating, was placed in a 5-ml brown volumetric flask, and was diluted with the mobile phase for HPLC as described below. Simultaneous determination of sesamol and tocopherol homologues in the oils[25] was carried out by using a Shimadzu LC-6A HPLC (Kyoto, Japan), equipped with a Shim-pack CLC-SIL (M) column (5 μm, 150 mm × 6.0 mm id, Shimadzu). The mobile phase was a mixture of *n*-hexane and ethyl acetate (90 : 10, v/v). Sample solution (20 μl) was injected with a fully loaded 20 μl loop. Antioxidants were monitored with a fluorescence detector (Shimadzu RF-535) set at an excitation wavelength 290 nm and emission wavelength 320 nm, and were quantified by comparison to the content before microwave heating.

Statistical analysis

Each reported value is the mean of two measurements from two replicates. To illustrate the relative stability of antioxidants during microwave heating, the values before treatment were normalised to 100. The data were subjected to one-way analysis of variance with a randomised complete block design to partition the effect of different parameters.[27] Significant differences among treatment means were separated by using Duncan's multiple range test, at a level of $P < 0.05$.[28]

RESULTS AND DISCUSSION

Tocopherol contents in commercially available vegetable oils were found in soya bean oil, 439.5 mg kg^{-1} (α, 59.2; β, 3.5; γ, 282.9; δ, 93.9), in rapeseed oil, 456.8 mg kg^{-1} (α, 149.4; γ, 294.0; δ, 13.4) and in safflower oil, 191.4 mg kg^{-1} (α, 180.0; γ, 6.0; δ, 5.4). However, no tocopherols were detected in the oils after purification by aluminium oxide column chromatography. These oils are termed purified vegetable oils in this paper. The purified oils contained no chlorophylls, free fatty acids or phospholipids (data not shown), and their chemical quality characteristics before microwave heating were as reported previously.[21] Table 1 gives the fatty acid compositions of purified oils before microwave heating. The fatty acid compositions of commercial and purified (tocopherol-stripped) oils were not significantly different ($P > 0.05$) from each other. The highest degree of unsaturation as calculated in Table 1[17] was shown by safflower oil (1.66), followed by soya bean (1.54) and rapeseed (1.41) oils. Observed sample (purified soya bean oil) temperatures at the end of 2450 MHz treatments are plotted (data not shown). The temperature of the oil increased sharply in the first 5 min of heating: ≈ 100 °C after 4 min heating, 170 °C after 8 min, 205 °C after 10 min and 210 °C at 25 min. In general, the frying conditions for

Fatty acid	Oil		
	Soya bean	Rapeseed	Safflower
14 : 0	0.1	0.1	0.2
16 : 0	11.3	4.0	9.3
16 : 1	0.1	0.2	0.1
18 : 0	3.3	1.6	1.8
18 : 1	23.6	58.9	12.0
18 : 2	54.0	23.4	76.1
18 : 3	7.5	11.5	0.5
22 : 1	0.0	0.3	ND
Saturates	14.8	5.7	11.3
Unsaturates	85.2	94.3	88.7
Degree of unsaturation[c]	1.56	1.61	1.66

[a] Each value is an average of three determinations and expressed as wt% of total fatty acid methyl esters.
[b] ND = 0.01%.
[c] Degree of unsaturation is calculated as [% palmitoleic + % oleic + % erucic + 2 × % linoleic + 3 × % linolenic] ÷ 100.

Table 1. Fatty acid composition of tocopherol-stripped vegetable oils before microwave heating[a]

fried foods, such as french fries,[29] are about 160–180 °C for 3.5 min and correspond to the 8–12 min heating in this study. There were no significant differences ($P > 0.05$) in temperatures among the oils containing added antioxidants.

In the first experiment, the oxidation of purified soya bean oil during microwave heating, after the addition of tocopherol or sesamol at 800 ppm, was determined by peroxide, carbonyl and anisidine value measurements (Fig 1). All antioxidants were effective in stabilising the substrate oil, and an increase of peroxide values was significantly ($P < 0.05$) inhibited by their additions. Sesamol was the most effective in stabilising substrate oil, followed by δ- or γ- and α-tocopherols in a decreasing order. α-Tocopherol is reported to have antioxidant activity at low concentrations but prooxidant activity at high concentrations.[30] When purified soya bean oil was heated in a microwave oven, the longer the microwave heating time, the greater became the carbonyl and anisidine values, as secondary indicators of oxidative deterioration. However, no appreciable change ($P > 0.05$) in anisidine value was observed up to 8 min of heating, but values changed rapidly from 12 to 16 min of heating. All antioxidants suppressed the formation of anisidine-reactive substances and the efficiency decreased in the order sesamol > δ- > γ- > α-tocopherols. The relative stability of sesamol and tocopherols in purified soya bean oil was compared during microwave heating (Fig 2). A significant change ($P < 0.05$) after microwave treatment was observed between α-tocopherol or sesamol and γ- or δ-tocopherol, respectively. The highest reduction rate was seen in α-tocopherol, followed by sesamol, while the reduction rate of γ- or δ-tocopherol was almost the same, and over 90% of their original levels was still retained after 25 min of heating.

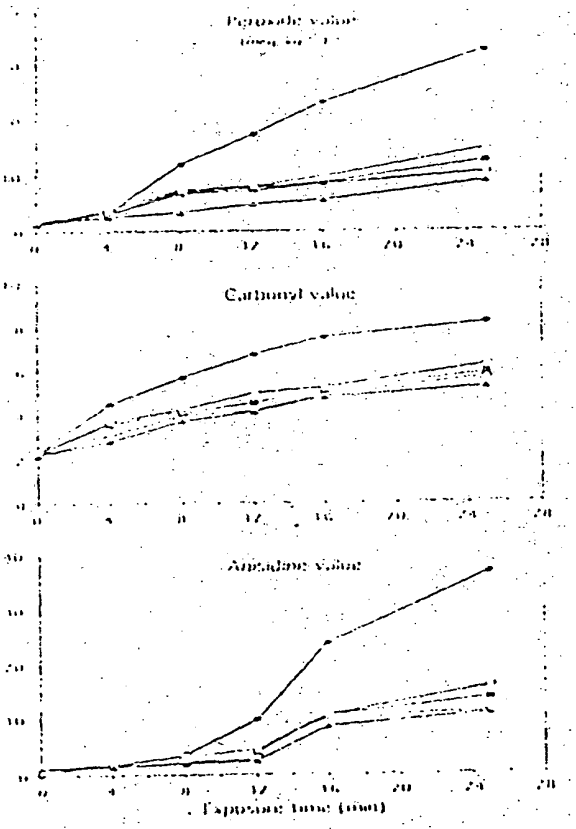

Figure 1. Effects of tocopherols or sesamol at 800 ppm levels on chemical characteristics of purified soya bean oil during microwave heating. ○, control; ▲, α-tocopherol; □, γ-tocopherol; ◇, δ-tocopherol; △, sesamol. All data points represent the means of two measurements from two replicates, and the standard errors are within the size of the symbols.

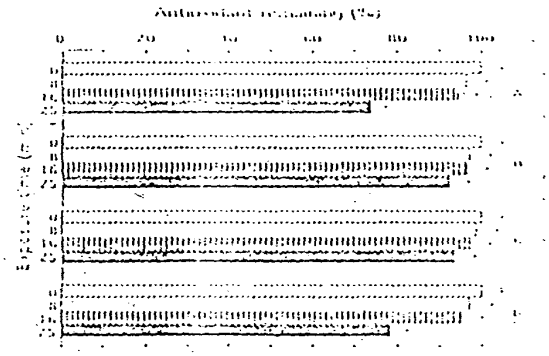

Figure 2. Effects of microwave heating on loss of tocopherols or sesamol at 800 ppm levels in purified soya bean oil. A, α-tocopherol; B, γ-tocopherol; C, δ-tocopherol; D, sesamol. Each value represents the average of three replicates and horizontal bars represent standard error of the replicates.

In the second experiment, to clarify the antioxidant effects in purified rapeseed oil during microwave heating, tocopherol or sesamol, or their mixtures (1 : 1, wt/wt) were added to the substrate oil at a total level of 400 ppm. Figure 3 shows the effects of the antioxidants on chemical characteristics during microwave treatments. The development of hydroperoxides, carbonyl and anisidine-reactive compounds during microwave heating was significantly ($P < 0.05$) inhibited not only by the addition of tocopherol or sesamol, but also that of their mixtures. The highest antioxidant activity was observed in the mixture of γ-tocopherol (200 ppm) and sesamol (200 ppm), followed by sesamol or γ-tocopherol at 400 ppm. A combination of α-tocopherol and sesamol was not as effective as the addition of the other antioxidants. The results suggest that sesamol has a synergistic action with γ-tocopherol as described previously.[11] Figure 4 illustrates the γ- or α-tocopherol and sesamol stabilities at 800 ppm or a combination of 200 ppm and 200 ppm in the purified rapeseed oil during microwave heating. When α-tocopherol was added to the substrate oil with sesamol (Fig. 3), it was consumed to a greater extent than sesamol during microwave heating (Fig. 4, upper B). However, the highest relative stability alone or in mixtures was seen with sesamol (Fig. 4, lower A, C), followed by γ- and α-tocopherols in a decreasing order (Fig. 4, upper). After 25 min of heating, sesamol or γ- and α-tocopherols were still retained at over 85 or 82 and 70% of the original levels, respectively. These trends do not correspond with those for a simple addition of the individual antioxidants at 800 ppm to purified soya bean oil (Fig. 2). The results with rapeseed oil indicated that α-tocopherol was consumed more rapidly, followed by γ- or δ-tocopherol, and that sesamol was consumed more slowly. In general, α-tocopherol would be expected to react more quickly with peroxide radicals produced in the oils than the other antioxidants. Similar trends have been reported[11] using lard or tocopherol-stripped corn oil.

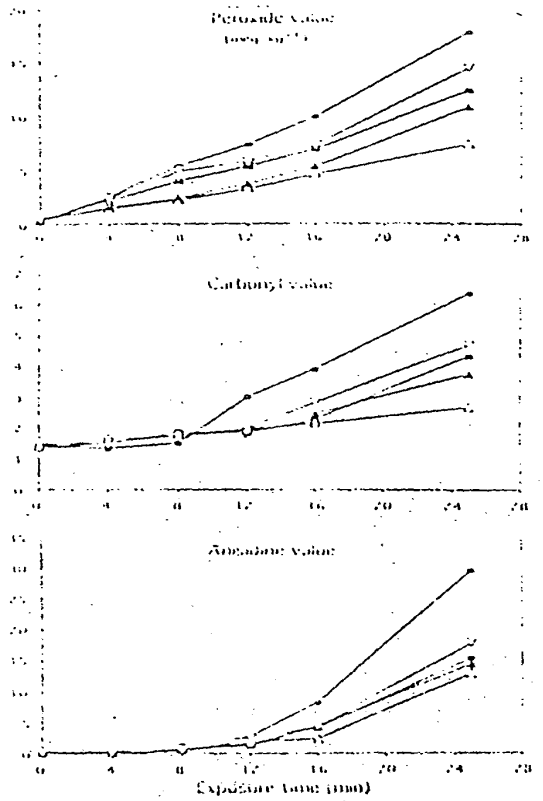

Figure 3. Effects of tocopherols and/or sesamol on chemical characteristics of purified rapeseed oil during microwave heating. ●, control; □, γ-tocopherol (400 ppm); ▲, sesamol (400 ppm); ▽, α-tocopherol (200 ppm) + sesamol (200 ppm); △, γ-tocopherol (200 ppm) + sesamol (200 ppm). All data points represent the means of two measurements from two replicates, and the standard errors are within the size of the symbols.

The addition of 50 ppm sesamol has been demonstrated to enhance the antioxidative action of γ-tocopherol at concentrations of 50–400 ppm in linoleic acid, it being especially strong at 400 ppm.[11] Also, the γ-tocopherol and sesamol contents in roasted sesame seed oil have been measured previously at levels of 400 ppm and 50–100 ppm, respectively.[8] Considering these reports from a practical point of view, the amount for the addition to purified oils was decided as follows: 50, 100 or 400 ppm for γ-tocopherol; 50 or 400 ppm for sesamol. Figure 3 shows the effects of a combination of γ-tocopherol and sesamol on the chemical characteristics of purified soya bean oil during microwave treatments. The quality characteristics of the oil during microwave heating were more significantly improved ($P < 0.05$) by a mixture of γ-tocopherol (400 ppm) and sesamol (400 ppm) than was observed by an addition of single antioxidants (800 ppm) (Fig. 1).

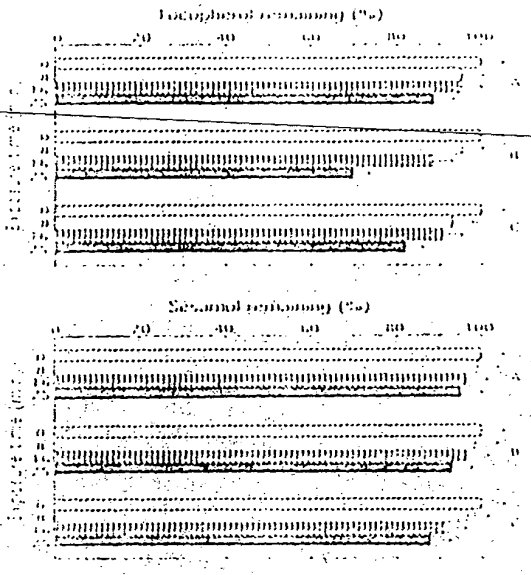

Figure 4. Effects of microwave heating on loss of tocopherols or sesamol and their mixtures at different concentrations in purified soybean oil. A, γ-tocopherol (400 ppm) or sesamol (400 ppm); B, γ-tocopherol (200 ppm) + sesamol (200 ppm); C, γ-tocopherol (300 ppm) + sesamol (300 ppm). Each value represents the average of three replicates and horizontal bars represent standard error of the replicates.

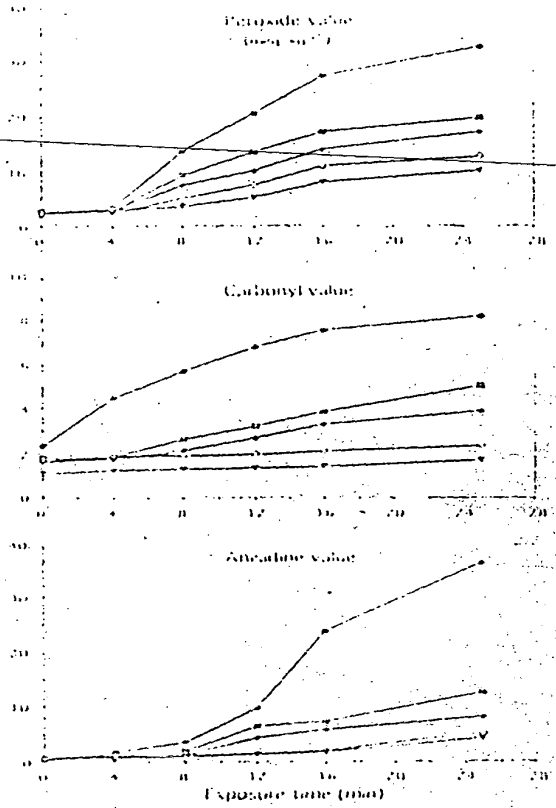

Figure 5. Effects of γ-tocopherol and/or sesamol on chemical characteristics of purified soya bean oil during microwave heating. ○, control; □, γ-tocopherol (50 ppm) + sesamol (50 ppm); ◇, γ-tocopherol (100 ppm) + sesamol (50 ppm); △, γ-tocopherol (400 ppm) + sesamol (50 ppm); ▽, γ-tocopherol (400 ppm) + sesamol (400 ppm). All data points represent the means of two measurements from two replicates, and the standard errors are within the size of the symbols.

The relative stability of γ-tocopherol and sesamol during microwave heating was compared at different concentrations in purified soya bean oil. Figure 6 illustrates a typical changing pattern of γ-tocopherol or sesamol stability at different concentrations (50 ppm to 400 ppm) in the oil following microwave heating. A significant change ($P < 0.05$) in γ-tocopherol was observed after microwave heating, and the change depended on the amounts of γ-tocopherol. The greater the tocopherol levels, the less was the percentage loss of tocopherol (Fig. 6, upper A, C), but the greater the actual loss in ppm (Table 2). Also, with the longer exposure to microwave energy, the percentage loss became significantly smaller ($P < 0.05$) with increased levels of tocopherol; at 400 ppm, over 80% of the original levels was still retained after 25 min of heating. However, the lower the level of tocopherol added to purified soya bean oil, the greater was the reduction in the percentage of tocopherol. At 50 ppm, γ-tocopherol was reduced to 70% of the initial level

Unheated Antioxidant		Loss after microwave heating					
		5 min		16 min		25 min	
γ-Toc	Sesamol	γ-Toc	Sesamol	γ-Toc	Sesamol	γ-Toc	Sesamol
400	400	8.0	0.0	48.0	12.0	68.0	28.0
400	50	20.0	1.5	48.0	3.0	64.0	5.5
100	50	6.0	2.5	18.0	9.0	25.0	10.0
50	50	13.5	6.0	15.0	10.0	16.0	11.5

Table 2. Loss of γ-tocopherol and sesamol (ppm) in purified soya bean oil during microwave heating[a,b]

[a] Each value is an average of two determinations. The content in loss of each sample was calculated from Fig. 6.
[b] Toc: Tocopherol.

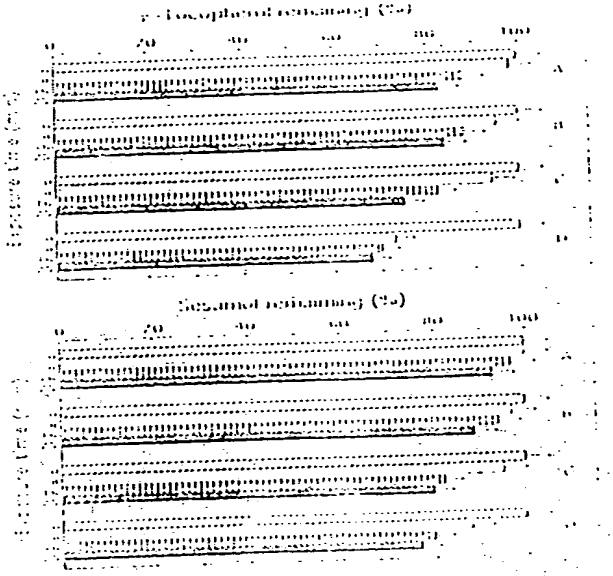

Figure 6. Effects of microwave heating on loss of γ-tocopherol or sesamol in the mixtures at different concentrations in purified soya bean oil. A, γ-tocopherol (400 ppm) + sesamol (400 ppm); B, γ-tocopherol (400 ppm) + sesamol (50 ppm); C, γ-tocopherol (50 ppm) + sesamol (50 ppm); D, γ-tocopherol (50 ppm) + sesamol (50 ppm). Each value represents the average of three replicates and horizontal bars represent standard error of the replicates.

after 8 min of heating and, thereafter, was retained at almost constant levels (98%). The relative stability of sesamol during microwave heating was also compared at 50 and 400 ppm in purified soya bean oil (Fig 6, lower A, C). A significant change ($P < 0.05$) in sesamol was observed after heating, but the actual loss in ppm was smaller at both levels than that of γ-tocopherol (Table 2). The results suggested that γ-tocopherol may be preferentially consumed during microwave heating in comparison with sesamol, when added as a combination of these antioxidants. The overall antioxidant activities of tocopherol and sesamol depend on their hydrogen-donating ability, relative stability, and distribution in purified oil. Namely, sesamol (mol wt 138) will contribute about a three times greater autoxidation chain-breaking OH function compared to the tocopherol (mol wt 416). Therefore, further studies are needed to clarify quantitatively the antioxidative activity of sesamol and tocopherols.

Figure 7 shows the effects of a combination of γ-tocopherol and sesamol on peroxide and anisidine values of purified safflower and rapeseed oils during microwave heating. Their additions delayed significantly ($P < 0.05$) increases in peroxide and anisidine values during heating. There were significant differences ($P < 0.05$) based both on the substrate oils and on the levels of antioxidants. Unlike α-tocopherol, γ-tocopherol has increasing antioxidant activity at higher concentrations.[24] There were significant differences ($P < 0.05$) in the anisidine values among the three purified oils after 16 min of the heating (Figs 5 and 7), especially because of the differences in their unsaturated fatty acids (linoleic or linolenic) Table 1). These differences may be attributed to the differences in secondary oxidation products such as aldehydes, alcohols, ketones, acids and lactones,[33,34] because the anisidine is particularly sensitive to the presence of aldehydes. Differences in anisidine value between the three purified oils became less pronounced ($P < 0.05$) when the γ-tocopherol concentration was increased from 50 ppm to 400 ppm. The relative stabilities of γ-tocopherol and/or sesamol in purified safflower and rapeseed oils during microwave treatments were omitted because they were essentially the same as those in purified soya bean oil (Fig 6).

The effectiveness of tocopherols as lipid antioxidants has been attributed mainly to their ability to break chain reactions by reacting with fatty acid peroxy radicals. Barton and Ingold[14] reported that the rate of scavengers for peroxy radicals by β- and γ-tocopherols was two-thirds, and δ-tocopherol one-fourth, that of α-tocopherol. However, the results obtained from this study are not necessarily in agree-

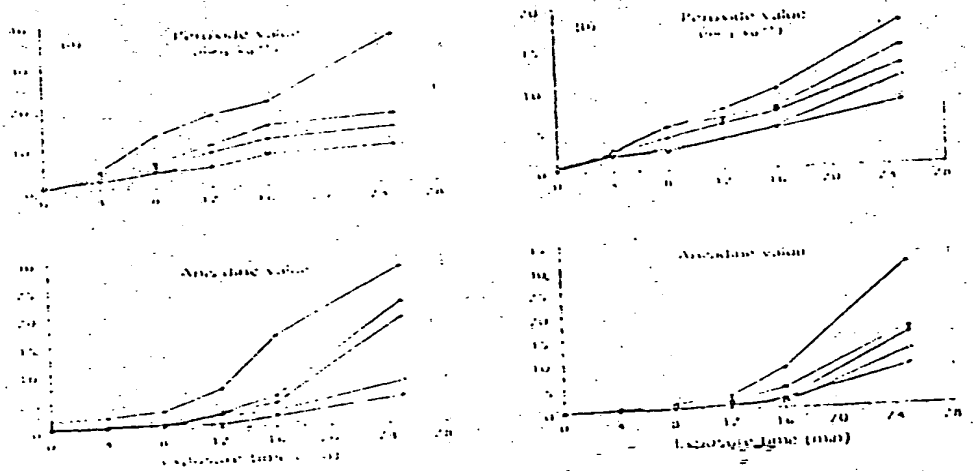

Figure 7. Effects of γ-tocopherol and sesamol on peroxide and anisidine values of purified safflower and rapeseed oils during microwave heating. (a) safflower oil; (b) rapeseed oil. ○, control; □, γ-tocopherol (50 ppm) + sesamol (50 ppm); ◇, γ-tocopherol (100 ppm) + sesamol (50 ppm); △, γ-tocopherol (200 ppm) + sesamol (50 ppm); ▽, γ-tocopherol (400 ppm) + sesamol (50 ppm). All data points represent the means of two measurements from two replicates, and the standard errors are within the size of the symbols.

ment with these abilities because of the differences in experimental conditions such as microwave heating and levels of addition (which are extreme importance for the effectiveness of γ-tocopherol which can become pro-oxidative at higher concentration).

CONCLUSIONS

The oxidative deterioration of purified substrate oils was significantly ($P < 0.05$) inhibited during microwave heating, by not only the addition of sesamol or tocopherols, but also that of mixture of these antioxidants. Very effective combinations of tocopherols and sesamol as antioxidants in the oils were 200 or 400 ppm for γ-tocopherol and 50, 200 or 400 ppm for sesamol, respectively. The overall antioxidant activities of individual tocopherols and sesamol or their mixtures depend on their hydrogen-donating ability, relative stability, and distribution in purified oils. Also, it is important to know the possibility of synergistic antioxidative action between tocopherol homologues and sesamol in various food systems.

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(9)

PRINCIPLES OF FOOD SCIENCE

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PART I

FOOD CHEMISTRY

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errors, especially in dry systems, since malonaldehyde reacts readily with free amino groups during nonenzymic browning.

5. OTHER METHODS

Determination of weight gained due to oxygen absorption and reaction has been used to measure stability of some fats or oils. Measurements of oxygen uptake by manometric techniques are useful for some systems, especially for relating the amount of oxygen absorbed to other measurable criteria such as peroxide value, diene conjugation, or the amount of carbonyl compounds. The spectrophotometric analysis of conjugated double bonds reflects early changes during oxidation of dienes, trienes, and other polyunsaturated fatty acids.

B. Lipolysis

The ester linkages of lipids are subject to hydrolysis resulting from enzymes, from thermal stress, or from chemical action. These reactions are collectively known as lipolysis, lipolytic rancidity, or hydrolytic rancidity.

Lipolysis of milkfat has been studied intensively because of the ease with which it occurs in raw milk and its importance to the flavors of various milk products. The common notion that butyric acid is responsible for the flavor of rancid milk has been disproved. All of the even-numbered fatty acids from C_4 to C_{12} contribute to rancid flavor, with no single acid having a dominant influence [23,24].

Lipolysis, regardless of the cause, seriously degrades the quality of cooking and frying fats. As a result of lipolysis, the smoke point (temperature at which vapor (smoke) can be seen in a beam of light over the surface of a heated fat) is severely depressed and fried foods, such as fried cakes and doughnuts, exhibit cracked surfaces, increased tendency to brown, and increased fat absorption. Small amounts of free fatty acids lower the smoke point to objectionable levels (see Table 4-17).

Free fatty acids that develop during storage and processing of oil seeds and animal tissues must be removed by refining processes and deodorization to yield fats and oils of acceptable quality. The resulting yield and cost of processing are of economic importance.

TABLE 4-17

Relation between Smoke Point and Free Fatty Acid Content of Cottonseed Oil

FFA (%)	Smoke point (°F)
0.01	450
1.0	320
10.0	260
100.0	200

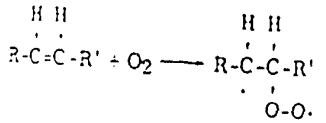
C. Autoxidation

Fats also can become rancid as a consequence of oxidation and this "oxidative rancidity" is a major cause of food deterioration. Lipolytic rancidity usually poses less of a flavor problem than oxidative rancidity since the former develops off flavors only in those fats which contain short-chain fatty acids (less than C_{12}).

Energy in the form of heat, light, or ionizing radiation or catalysis by prooxidant metals or enzymes contribute to the oxidation process. Chemical oxidants, when present, also oxidize lipids.

1. MECHANISMS OF OXIDATION

The reaction of oxygen with unsaturated fatty acids in lipids constitutes the major means by which lipids or lipid-containing foods deteriorate. Oxidation of fat is frequently alluded to as autoxidation because the rate of oxidation increases as the reaction proceeds. Unless mediated by other oxidants or enzyme systems, oxidation proceeds through a free-radical chain reaction mechanism involving three stages: (1) initiation, formation of free radicals; (2) propagation, free-radical chain reaction; and (3) termination, formation of nonradical products. Hydroperoxides are the major initial reaction products of fatty acids with oxygen. Subsequent reactions control both the rate of reaction and the nature of products formed. In the initiation stage an unsaturated hydrocarbon loses a hydrogen to form a radical, $RH \rightarrow R\cdot + H\cdot$, and oxygen adds at the double bond to form a radical:



Alternatively, oxygen in the singlet state can apparently interpose between a labile hydrogen to form a hydroperoxide directly ($RH + O_2 \rightarrow ROOH$). The latter may be a special case and is referred to here only briefly. Direct formation of hydroperoxides is not necessarily a free-radical chain mechanism, although it can initiate chain processes.

During propagation, the chain reaction is continued by $R\cdot + O_2 \rightarrow ROO\cdot$ and $ROO\cdot + RH \rightarrow ROOH + R\cdot$ to form peroxy radicals, hydroperoxides, and new hydrocarbon radicals. The new radical formed then contributes to the chain by reacting with another oxygen molecule.

When two radicals interact, termination occurs:

$$R\cdot + R\cdot \rightarrow RR$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$$

$$RO\cdot + R\cdot \rightarrow ROR$$

$$ROO\cdot + R\cdot \rightarrow ROOR$$

$$2\,RO\cdot + 2\,ROO\cdot \rightarrow 2\,ROOR + O_2$$

β crystals. Temperatures different from those at which transformations of different sets of mixed crystals, giving melting over a wide range of temperature.

The rates at which polymorphic transformations occur depend on the number of polymorphic forms into which the fats can change. Triglycerides exhibit relatively limited number of different types of triglyceride molecules exhibit few polymorphic forms and tend to change rapidly into the β form. Fats such as lard flakes, soybean oil flakes, and cocoa butter behave in this manner. Fats with greater randomness of interesterification in the triglyceride molecules exhibit polymorphic forms and transform relatively slowly into the β form. Hydrogenated lard, ordinary lard, and hydrogenated cottonseed oil exhibit the polymorphic forms β'-2 and β, while lard exhibits four forms, α, β'-2, β'-1, and β. Hydrogenated cottonseed oil flakes can go through the same forms, and β'-1 and β. Commercial mixed fats, such as vegetable shortening, hard fats, frying fats, and partly hydrogenated vegetable oils usually exhibit α, β'-2, β'-1, and β and form β crystals.

Tempering time can be reduced by maintaining fat at a temperature just below the melting point of its most stable form. Intermittent crystallization of the fat mass is facilitated by agitation and by the presence of fine crystals. Rapid formation of additional small crystals of the most stable form. The β' form is most stable form. The β'-2 form is more stable than the β'-1 form. The β'-2 form is more stable than the β'-1 form. Fats from hydrogenation of fats and oils are usually tempered to the most stable form by temperature procedures.

VI. CHEMICAL PROPERTIES AND DETERIORATION

A. Stability and Its Characteristics

Stability refers to the capability of a fat, oil, or fatty food to maintain a fresh taste and odor during storage and use. It is related to the degree of unsaturation of the lipid moiety, the nature and location of stresses in the system, the presence or absence of pro-oxidants or anti-oxidants, and the environmental conditions. Fats with substantial unsaturation in the fatty acids are readily oxidized to form relatively unstable and flavor-containing products reflect this instability. Vegetable oils usually tend to be more stable than some of the animal fats, such as lard, even though the total unsaturation of the vegetable oils may be greater. This can occur because natural anti-oxidants are usually present in the vegetable oils.

A challenge has long existed to develop methods which are accelerated in time of execution but which accurately portray the length of time a fat or food remains palatable under conditions encountered during marketing. Most of these methods involve elevation of temperature with or without increased exposure to oxygen, and a suitable objective method of evaluation. Some methods for evaluating the quality of fats are discussed below.

1. ACTIVE OXYGEN METHOD

The active oxygen method (AOM) involves bubbling air at a controlled rate through a sample of fat maintained at 100°C. Rancidity is determined by sniffing the effluent air periodically. Correlations have been made between the development of peroxides and the first presence of rancidity, rapidly developing rancidity. A trained observer can detect rancidity in lard at a peroxide value of about 20 in hydrogenated oils at a value of about 100 in vegetable oils such as cottonseed and soybean, at a value of 100. [illegible] peroxide values [illegible] Peroxide values periodically and [illegible] of fats and oils. The time in hours to the [illegible] peroxide value is then interpolated and recorded. This method is widely used to determine the relative stability of fats and oils with the addition of antioxidants. However, attempts to correlate AOM values with the keeping quality of fats and oils in ambient storage generally have been unsuccessful.

2. OVEN STABILITY METHOD

The oven test, also known as, commonly called the Schaal oven test, involves holding the fat or oil sample in shallow containers at 63°C. Samples are sniffed at intervals to [illegible] the onset of rancidity. The relative stability of the fat or oil [illegible] is then determined by comparing [illegible] extremely [illegible].

3. [illegible] TEST

The [illegible] method involves placement of a sample in a bottle, the addition of a fat, [illegible] of oxygen, and the monitoring of pressure at constant temperature. The time at which oxygen uptake accelerates, as determined by a decrease in pressure, corresponds to the stability of the sample. This method is useful for measuring the relative effectiveness of antioxidants in prolonging the keeping time of fresh foods such as potato chips.

4. CARBONYL DETERMINATIONS

Extraction [illegible] and measurement of carbonyl compounds which have formed provides an indication of the stability of fats in some foods, but the results are unreliable in systems containing components which react readily with aldehyde. The method is further complicated by different responses of aldehydes formed from different fats or oils, and by different responses occasioned by the kind of hydroperoxide scission reaction involved. In addition to peroxides, measurements of saturated aldehydes, enals, dienals, and dialdehydes must be made to provide a picture of the oxidative state of the lipid or the food in which it occurs.

Acid stability is unsuitable for some systems since it, or the precursors, develops in meat and fish systems in proportion to the elapsed time for incipient rancidity and the intensity of rancidity. This method is also subject to man

Antioxidant Activity of Oat Extract in Soybean and Cottonseed Oils

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A previously published method for extracting antioxidants from Noble oats with methanol was modified to improve the antioxidant activity. The extract was tested in soybean and cottonseed oils held at 30 and 60°C in the dark and at 30°C in the light. During storage, the peroxide values (PV) of the oils were generally significantly lower ($P \leq 0.05$) with the addition of the extract than was the control (no additives), and the PV were slightly higher than for oils containing TBHQ. In addition, the extract was added to emulsions of the same oils and held at 30°C in the light and at 60°C in the dark. The PV of the emulsions containing the extract were significantly lower ($P \leq 0.05$) than were the PV of those containing tertiary butylhydroquinone and the control.

KEY WORDS: Antioxidant, autoxidation, cottonseed oil, oat, oxidation, soybean oil.

The addition of antioxidants to fats and oils or to foods that contain fats and oils is one of the most efficient ways to prevent oxidation of the lipids. There is a concern about the possible toxicity of synthetic antioxidants (1), so the popularity of natural antioxidants has increased. Although there is no assurance of the safety of natural antioxidants, there is some comfort knowing that such antioxidants were purified from natural products that have been consumed for generations.

The utilization of natural antioxidants from oat was first reported by Musher (2–5). The ground or aqueous extracts of cereals, including oat, and oilseeds effectively prevented lipid oxidation at both room and accelerated room temperatures (2–5). Musher (4) claimed that oat flour increased the stability of oils, fats, margarine and mayonnaise. The oat flour also was effective when dusted over bacon and potato chips.

Daniels and Martin (6) isolated and purified ferulic and caffeic acids from oat. The antioxidant activity of an oat extract containing these phenolics was as effective as propyl gallate (PG) and butylated hydroxytoluene (BHT) as measured by a recording oxygen apparatus (6). Further work showed that the extract could be separated into 24 active fractions by thin-layer chromatography (TLC) and column chromatography (CC). Some of the fractions were identified as caffeic and ferulic acid esters and monoesters of C_{26} and C_{28}, α,ω-diols (6), and monoesters of hexacosan-1-ol, 26-hydroxyhexacosanoic acid and 28-hydroxyoctacosanoic acid (7–9). Glycerol monoesters of 26-hydroxyhexacosanoic acid and 28-hydroxyoctacosanoic acid also were found (8,9). Collins *et al.* (10,11) found a group of cinnamic acid conjugates, namely avenanthramides. The structure of 10 components in this group were elucidated by TLC and CC, mass spectrometry (MS), nuclear magnetic resonance (NMR) and ultraviolet absorption spectroscopy (UV).

Solvent extraction is the major method used to isolate natural antioxidants. Supova *et al.* (12) reported that various solvent extracts of oat had antioxidant activity in lard when tested by the active oxygen method. Also, solvents with higher polarity yielded greater antioxidant activity, and a methanolic extract of defatted oat flour was the most active. Chang *et al.* (13), in a patent for extraction of antioxidants from rosemary and sage, found methanol and ethanol to be the most successful solvents. Duve and White (14) compared the activity of eight solvent extractions of oats and concluded that the greatest antioxidant activity was derived from the methanol extracts of undefatted oat.

The objectives of the current study were threefold. The first was to determine an improved method to extract and concentrate the antioxidants from oat. The second was to verify the presence of phenolic antioxidants as the active components in the extract by TLC, gas chromatography (GC) and by GC/MS. The third objective was to test the antioxidant activity of the extract in soybean and cottonseed oils and their emulsions under different storage conditions.

EXPERIMENTAL PROCEDURES

Extraction of oat antioxidants. Noble oats (*Avena sativa* L.) were grown near Ames, Iowa, in 1991 and 1992. After harvest, the dehulled oat groats were stored at 4°C and 45% relative humidity until needed for extraction. The groats were ground, and the resulting powders were passed through a No. 40 U.S. standard mesh screen.

The extraction of antioxidants and Δ^5-avenasterol from oat was done by the method of Duve and White (14) with some modifications. As previously described (14), oat flour (1 kg) was extracted with methanol (4 L) at room temperature with constant stirring for 24 h. The solvent was changed every day for seven days. At every solvent change, the mixture was filtered through Whatman #4 filter paper. The filtrates were combined and evaporated in a rotary evaporator to 30 mL at 45°C.

Modification of the method of Duve and White (14) involved fractionating the crude extract through a silicic acid column (15 mm × 220 mm). The silicic acid (100 mesh; Aldrich Chemical Company, Milwaukee, WI) was activated overnight at 120°C, then washed first with 400 mL methanol three times and then with 400 mL hexane three times. A hexane slurry of the treated silicic acid (200 g) was then packed into the column, and 15 mL of methanolic extract was applied to the top. The column was eluted stepwise with 500 mL hexane and 500 mL methanol. The two separated fractions were then rotary-evaporated to 30 mL each at 45°C and stored under nitrogen at −10°C until analyzed.

Determination of total phenolic contents (TPC). TPC of the extract was tested by Method (9.110) of the Association of Official Analytical Chemists (AOAC) (15). Briefly, oat extract (0.1 mL) was added into a 100-mL volumetric flask containing 75 mL distilled water. Folin–Denis reagent (5 mL) and saturated sodium carbonate solution (10 mL) were added to the flask and diluted to 100 mL with distilled water. The mixture was then shaken for 1 min and allowed to stand at room temperature for 30 min. The absorbance of the solution was viewed in a spectrophotometer at 760 nm.

Oil storage tests. All storage tests were conducted with refined, bleached and deodorized soybean and cottonseed

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oils obtained from commercial sources. The oils contained no additives except citric acid. All tests were run on duplicate oil samples. Oat extract (0.005, 0.02 and 0.03%, wt/wt based on TPC of the extract placed in the oil) was partly dried under nitrogen first and then added to 10 g of cottonseed oil stored at 30°C for 30 d or at 60°C for 25 d in the dark. Oat extract at slightly different concentrations than just listed (0.01, 0.02 and 0.03%) was added to the cottonseed and soybean oils stored at 30°C under fluorescent light at a distance of 180 foot-candles (ft-c) for 10 d and to soybean oil stored at 60°C in the dark for 10 d. Additional treatments for each test included tertiary butyl hydroquinone (TBHQ) (0.02%), the best synthetic antioxidant available (16), and a control containing no additives (except citric acid). All treatments were stored in open beakers (50 mL) that had been precleaned with a potassium ethanol solution (5 g potassium hydroxide per 100 mL ethanol). The oils were sampled every two days.

Emulsion storage tests. The antioxidant activity of the oat extract also was tested in soybean and cottonseed oil emulsions. All tests were run on duplicate emulsion preparations. The emulsions consisted of 10 g (56%) oil blended with 7.7 g (43%) water and 1 g (1%) Tween 20, an emulsifier, (Aldrich). Oat extract (0.01, 0.02 or 0.03%) or TBHQ (0.02%) was added to each treatment mixture and blended in a Waring commercial blender (Dynamics Corporation of America, New Hartford, CT) for 1 min to form stable emulsions. A control treatment with no additives also was tested. The emulsions were then stored at 30°C under fluorescent light at a distance of 180 ft-c or at 60°C in the dark for up to 10 d. All treatments were stored in open beakers (50 mL) that had been pretreated with potassium ethanol solution as previously described. The emulsions were sampled every two days.

Peroxide values (PV). The PV of the oils were analyzed on the day of sampling by the Stumm test as modified by Hamm *et al.* (17). The PV of the emulsions were analyzed according to AOCS Standard Method cd 8-53 (18). Because the emulsions contained water, the Stumm test was not suitable for PV measurements. The PV of the oils and emulsions were run in triplicate and duplicate, respectively.

GC of fatty acid methyl esters (FAME). Soybean and cottonseed oils and emulsions from the storage tests were analyzed for fatty acid composition on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Kennett Square, PA) equipped with a flame-ionization detector and split/splitless injector. A DB-23 fused silica capillary column was used with dimensions of 0.25 mm × 15 m × 0.25 μm film thickness (J&W Scientific Inc., Rancho Cordiva, CA). Chromatographic parameters were set as follows: injector temperature, 250°C; detector temperature, 250°C; column temperature programming, 140 to 200°C at 12°C/min with 5°C min holding time at 200°C; and carrier gas (H_2) at 100 mL/min. The fatty acids were converted to FAME by following the procedure of Hammond and Fehr (19). All tests were run in duplicate, and the results were averaged.

TLC for antioxidant activity. The method of Pratt and Miller (20) was used to estimate the antioxidant activity of the oat extracts. The TLC plates (0.25 mm) precoated with silica gel G (Fisher Scientific, Itasca, IL) were activated at 120°C for 2 h. Oat extract (25 μL) was streaked on the plates and developed in the upper phase of chloroform/ethanol/acetic acid (98:2:2). After development, the plates were dried and sprayed with a β-carotene solution. The β-carotene (9 g) was dissolved in 30 mL chloroform and mixed with two drops of linoleic acid and 60 mL ethanol. The intensity of orange color corresponded to the antioxidant activity of the oat extract (20). During the preliminary stages, the ground groats, whole groats and hulls were extracted with methanol. The methanolic extract of ground groats had the best antioxidant activity; therefore, it was chosen for further study.

TLC to identify the chemical composition of the oat extract. The chemical composition of the oat extract was tentatively determined by following a modified procedure of Taga *et al.* (21). The purified extract (50 μL) obtained after CC, was streaked on a TLC plate (0.25 mm) and developed first in the solvent system of chloroform/ethanol/acetic acid. The plate was viewed under UV light (360 nm) and then sprayed with β-carotene along one side of the plate to identify bands of antioxidants. The clean portions of the separated bands (positive in β-carotene spray) were scraped from the plate, extracted in methanol and concentrated to 1.5 mL. The extracts of each band (10 μL) were spotted on another TLC plate and developed in *n*-butanol/acetic acid/water (4:1:5). Nine sprays were used to help identify the composition of the bands (Table 1) (22–30).

GC and GC/MS to identify the chemical composition of the oat extract. The oat extract was analyzed for its phenolic acid composition. The extract was first hydrolyzed and then derivatized with trimethylsilyl (TMS) by the method of Pometto and Crawford (31). The gas chromatograph was identical to that described for analysis of FAME. The MS was a Hewlett-Packard 5970 mass-selective detector. A SPB-1 fused-silica capillary column was used with dimensions of 0.25 mm × 25 m × 0.25 μm film thickness (Supelco Inc., Supelco Park, Bellefonte, PA). Chromatographic parameters were set as follows: injector temperature, 240°C; detector temperature, 260°C; column temperature programming, 120°C to 260°C at 12°C/min with 2 min holding at 140°C and 10 min holding at 260°C; and column flow, 1.74 mL/min.

The following standards (Sigma Chemical Company, St Louis, MO) were converted to TMS derivatives and then analyzed by GC and GC/MS (31): ferulic acid, caffeic acid, *trans*-cinnamic acid, *o*-coumaric acid, 3,5-dimethoxy-4-hydroxy coumaric acid, protocatechuic acid, syringic acid, syringaldehyde, gentisic acid, vanillin, *p*-hydrobenzoic acid, vanillic acid and 3,4-diethoxybenzoic acid. The GC/MS and retention time of unknown compounds in the extract were compared with those of the standards to verify identification.

Data and statistical analyses. All data are the average of two replicate experiments. The least-square means of the PV and individual fatty acid contents were calculated by the Statistical Analysis System (SAS) (32). The significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Preliminary storage tests with unmodified oat extract. Methanolic oat extracts, prepared exactly as described by Duvo and White (14), were added to soybean oil and stored. As before, there were no apparent difference

TABLE 1

Sprays Used in Antioxidant Identification by Thin-Layer Chromatography and Results for Bands Testing Positive

Sprays	Band	Color	Identified components (reference number)
$FeCl_3$–$K_3Fe(CN)_6$	A,B,C	Blue	Phenolics (22)
$FeCl_3$	A,B,C	Red/brown	Phenolics without *o*-/*p*-OH[a] (23)
NH_4OH–$AgNO_3$	B,C	Brown/black/gray	Reducing compounds (24)
Van-pts	A	Red-violet	Flavonoids phloroglucinol nucleus
	B,C	Pink	Flavonoids resorcinol nucleus (25)
DPNA[b]	A,B,C	Brown	Free *o*/*p*-OH phenolics[a] (26)
Na_2CO_3	B,C	Fluorescent color changes	Free OH phenolics (27)
I_2 vapor	A,B,C	Brown	Sugar mercaptals, alcohols, hexanoic acids, glycerides, *N*-acylamino sugars, neutral and acid polysaccharides (28)
Aniline oxalate	A	Green-brown	Hexoses
	B	Red	Pentoses
	C	Yellow	Uronic acids (29)
p-Anisidine-HCl	A,B	Light brown	Deoxysugars, aldohexoses
	C	Brown	Aldopentoses (30)

[a]Phenolics with free *ortho*- or *para*-hydroxy groups.
[b]Diazotized *p*-nitroanilino.

effectiveness among the treatments, containing extracts and the control with no additives, when stored at 60°C for up to 20 d. Data are not shown. Modifications of the extract as described in the Materials and Methods section revealed more promising antioxidative potential as measured by TLC, so the extract was further tested as described in this paper.

TPC tests. The TPC of each change of methanol from the oats was determined (Table 2). The first solvent change resulted in the greatest amount of phenolic material. Generally, the remaining phenolic content was reduced to half in each subsequent extraction. From the fifth to the seventh solvent change, the phenolic contents were very low. The TPC of the hexane eluant from CC of the crude extract also was tested, and only a small amount of phenolics was found. These results again confirmed that nonpolar solvents were not effective in extracting phenolics. The TPC of the methanolic eluant decreased slightly, from 30.5 ppm before CC to 30.1 ppm after CC. About 50% of the extract consisted of dry material, which included the phenolics and, likely, nitrogenous impurities (7). Preliminary tests revealed a decrease in antioxidant activity if the extract became dry. For example, the methanolic eluant from CC was evaporated to dryness and tested for TPC. A reduction in TPC of the extract occurred from 30 ppm before drying to 11 ppm after drying, likely because of the oxidative decomposition of phenolics when dried and exposed to air. Therefore, the extract always was stored in solvent and under nitrogen.

TLC for antioxidant activity. The TLC with β-caroten spray showed that the purified oat extract (after CC) produced a darker orange color than did the crude extract, suggesting that purified oat extract had better antioxidant activity than did the crude extract. No antioxidant activity was found in the hexane eluant of CC. The dried purified extract had little antioxidant activity, which agreed with the TPC results.

Identification of chemical components. The TLC and sprays described in Table 1 were used to tentatively identify the antioxidants present in the modified methanolic extract. Six bands were revealed under UV radiation with R_f values of 0.93, 0.81, 0.62, 0.53, 0.41 and 0.29. Three of these bands [R_f = 0.93 (A), 0.81 (B) and 0.29 (C)] tested positive with β-carotene spray. These three bands were tested with the sprays, and results are listed in Table 1.

Bands B and C had R_f values (0.81, 0.29) similar to those found by Duve and White (R_f = 0.80, 0.30) (14). Band A (R_f = 0.93) had a similar chemical content, but different R_f than did band B (R_f = 0.64) found by Duve and White (14). Perhaps the phenolics were bound by different numbers of chemical groups in the current and previous studies.

GC and GC/MS identification of phenolic acid composition. The presence of both ferulic and caffeic acids in the extract was confirmed by both GC and GC/MS.

Oil storage tests. No significant differences were found in FAME of any treatments during storage of soybean and cottonseed oils. In addition, the FAME of unsaturated fatty acids dropped little in all treatments by the end of the storage tests. Beginning FAME for soybean and cottonseed oils are shown in Table 3.

TABLE 2

Total Phenolic Content from Each Change of Methanol

Solvent changes	Phenolic content (ppm)[a]
1	16.97
2	7.89
3	4.25
4	1.41
5	0.67
6	0.24
7	0.10
Total	30.53

[a]The phenolics were extracted from 1000 g of oat groats.

TABLE 3

Fatty Acid Composition (relative area %) of Fresh Soybean and Cottonseed Oils

Oils	14:0	16:0	18:0	18:1	18:2	18:3
Soybean	—[a]	11.0	4.2	23.8	53.5	7.8
Cottonseed	1.0	24.1	2.4	17.5	53.9	—[a]

[a]<0.01%.

Figure 1 shows the results of cottonseed oil treatments stored at 30°C in the dark for 30 d. The PV of the control oil was significantly higher than PV of all other treatments after 8 d of storage. The treatments containing 0.005, 0.02 and 0.03% were not significantly different from each other until day 18, when the treatment containing 0.005% oat extract was significantly higher in PV than the other treatments containing oat extract. The oil containing 0.03% oat extract had a significantly lower PV than did the oil containing 0.02% oat extract on days 28 and 30. The treatment containing 0.02% TBHQ was not significantly different in PV from the oils containing different levels of oat extract until day 16, after which the treatment containing 0.02% TBHQ maintained a significantly lower PV than all other treatments. The magnitude of the differences between treatments that contained oat extract and the treatment containing 0.02% TBHQ, however, was not great.

When cottonseed oil was stored at 60°C in the dark for 26 d (Fig. 2), the control oil was significantly higher in PV than all other treatments from day 2 on. Until day 4, no significant differences were found among the treatments containing different levels of oat extract and TBHQ. The treatment containing 0.005% of oat extract had a significantly higher PV than treatments containing the other two levels of oat extract and TBHQ after 4 d of storage. From day 18 on, the treatment containing 0.02% oat extract had a significantly higher PV than did the treatments containing 0.03% oat extract and TBHQ; however, the treatment containing 0.03% oat extract had a significantly lower PV than did all other treatments after day 14.

During storage of cottonseed oil at 30°C in the light (Fig. 3), the control treatment was significantly higher in PV than were all other treatments. On day 2, the treatments containing 0.01 and 0.02% oat extract, which were not significantly different from each other, had significantly higher PV than did the treatments containing 0.03% oat extract and 0.02% TBHQ. There was no significant difference between the last two treatments. After day 2, the treatment containing TBHQ had the lowest PV followed by the treatments containing 0.03, 0.02 and 0.01% oat extract, respectively, which were all significantly different from each other.

Storage of soybean oil treatments at 60°C in the dark (Fig. 2) revealed no significant differences among the treatments that contained any level of oat extract or TBHQ. After day 2, the PV of the control oil were much higher than were PV of the rest of the treatments.

Figure 3 shows the results of soybean oil treatments stored at 30°C in the light. After day 4, the treatments that contained oat extract had significantly lower PV than did the control. The treatment containing 0.02% TBHQ

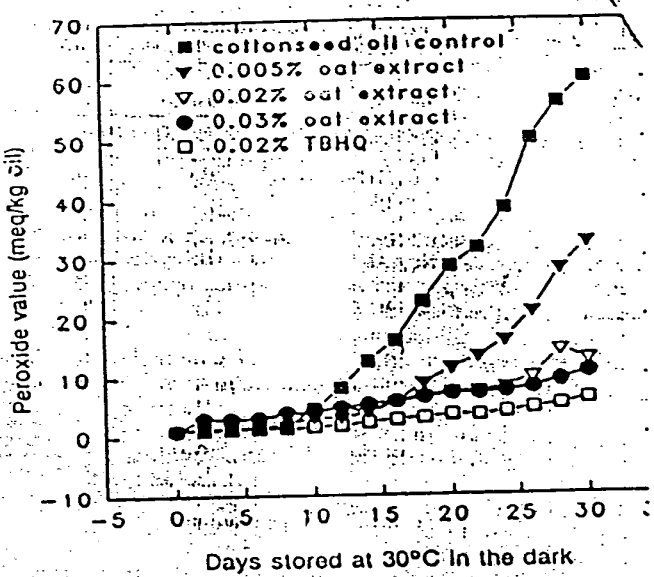


FIG. 1. Peroxide values of cottonseed oil treatments stored at 30° in the dark. TBHQ = tertiary butyl hydroquinone.

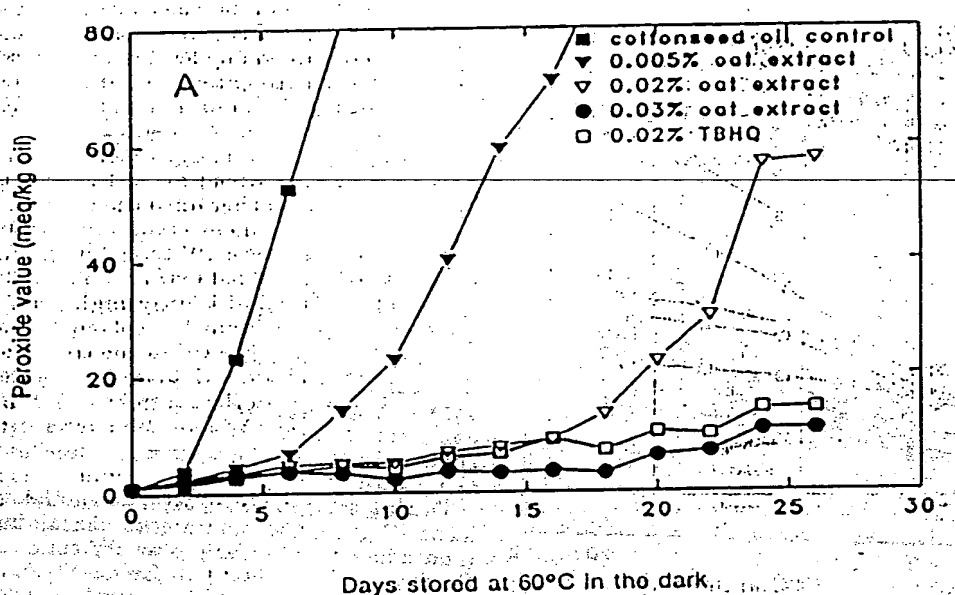
was significantly lower in PV than all other treatmen throughout storage. On days 2 and 4, the treatm nt co taining 0.01% oat extract was significantly lower in P than treatments containing the other levels of oat extrac But by days 6, 8 and 10, no significant differences wo found among the treatments that contained differe levels of oat extract.

Oat extract was similarly effective in both soybean ar cottonseed oils, even though the degree of unsaturatic in soybean oil was higher than in cottonseed oil (Table 3 The oat antioxidant tended to give better protection both soybean and cottonseed oils in the dark at accele ated room temperature (60°C) than did TBHQ (Fig. 2

At 30°C in the light and 60°C in the dark, the oat e tract at 0.005% was much less effective in cottonseed o than at 0.02 and 0.03%. Because no differences were fou within soybean oils containing oat extract at levels of 0.0 0.02 or 0.03% and cottonseed oil containing 0.02 or 0.03 oat extract, perhaps 0.01% oat extract was th minimu amount needed for maximum effectiveness.

In all storage tests, the induction periods of the oils co taining oat extract at all levels or TBHQ were much long than was the induction period of the control. Some a tioxidant treatments had not even reached the end of the induction period by the end of the storage test. For e ample, at 60°C in the dark (Fig. 2), the cottonseed oil co trol reached the end of its induction period in 2 d, where the treatments containing 0.03% oat extract or TBH had not reached the end of the induction period after 2 d of storage. The treatments containing 0.005 and 0.02 oat extract had induction periods of 8 and 18 d, respe tively. Other tests showed similar results.

The results in th current experiments are different fro those of Duve and White (14) who cited n significant d ferences in PV among soybean oil treatments that co



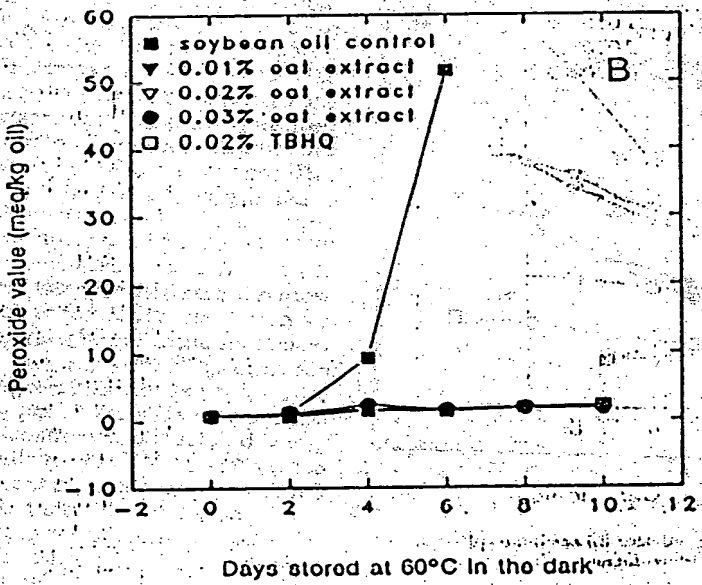
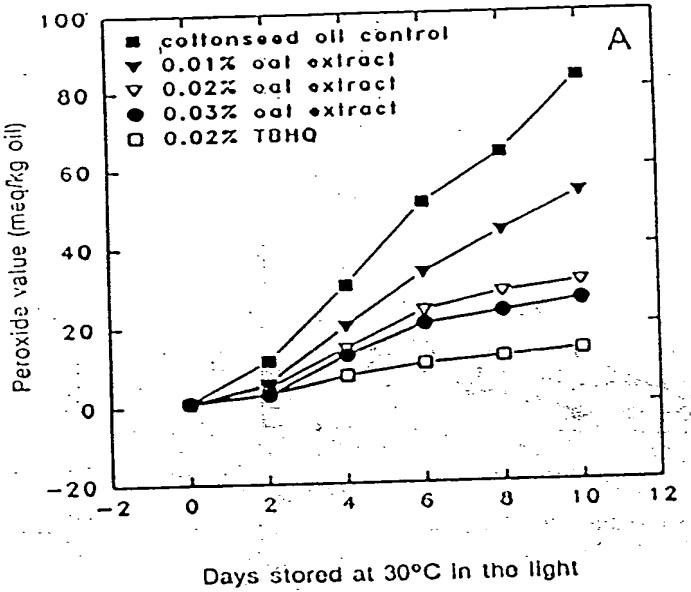


FIG. 2. (a) Peroxide values of cottonseed and (b) soybean oil treatments stored at 60°C in the dark. Abbreviation as in Figure 1.

tained oat extract and a control stored at 60°C for 20 d. But they did note some effectiveness of oat extract in soybean oil stored at 32°C in the dark for 80 d. The significant improvement of the oat extract in the current experiments is likely due to the higher purity of the oat extract obtained by the modified extraction procedure and because the amount of extract added in the current tests was based on total phenolic content and not just weight of the extract.

Emulsion storage tests. Th initial FAME of soyb an



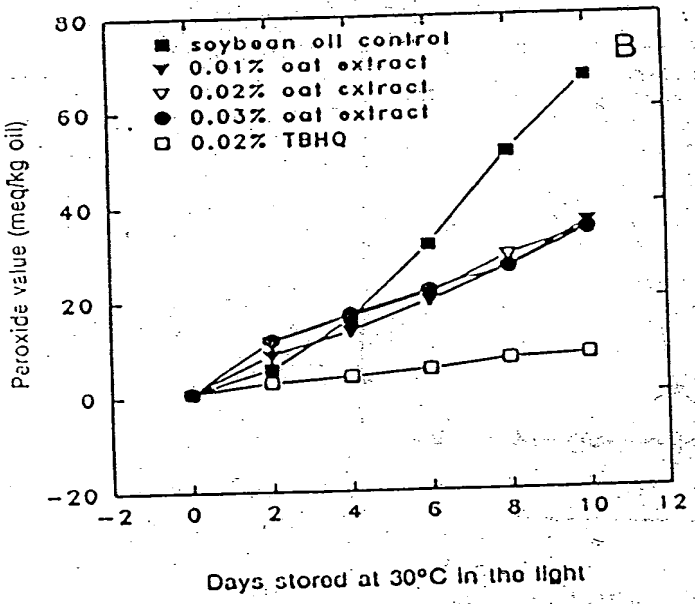


FIG. 3. (a) Peroxide values of cottonseed and (b) soybean oil treatments stored at 30°C in the light. Abbreviation as in Figure 1.

and cottonseed oils used in the emulsions were the same as those for the pure oils (Table 3). No significant differences in FAME were noted among treatments during storage, and the decreases in unsaturated fatty acids were small by the end of the storage tests, so these data are not shown.

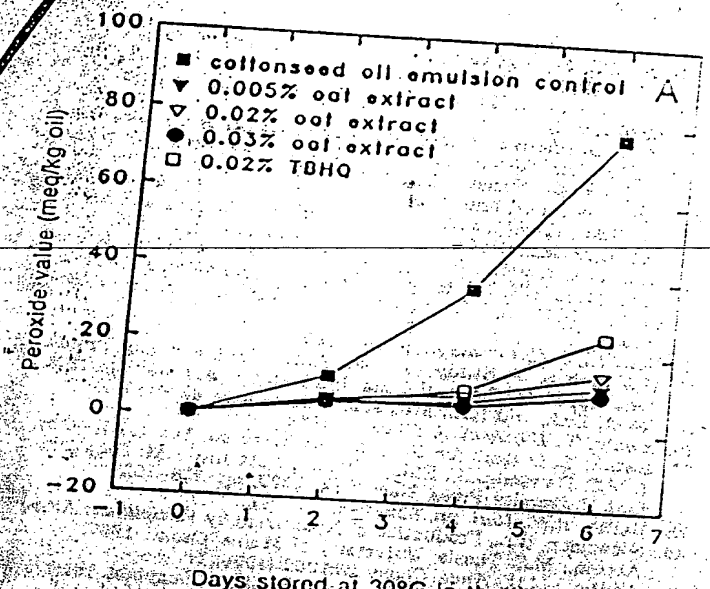
During 6 d of storage at 30°C in the light, the treatments that contained oat extract or TBHQ had significantly lower PV than did the control (Fig. 4). In general, no significant differences were observed among the treatments that contained any level of oat extract throughout the study. The treatment containing TBHQ had a significantly higher PV than did the treatments containing any level of oat extract on day 6.

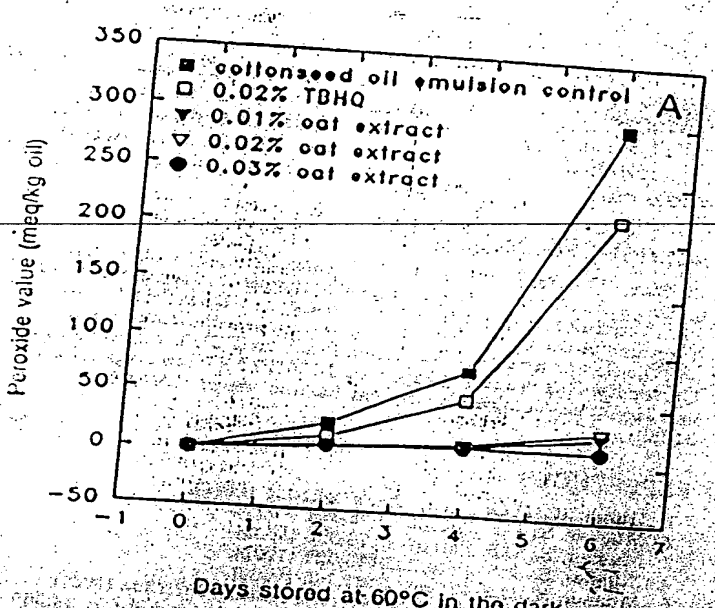
When stored at 60°C in the dark (Fig. 5), the treatments containing any level of oat extract were not significantly different from each other until the last day of storage, at which time the treatment containing 0.03% oat extract had a significantly lower PV than did the treatments with the other two levels of oat extract. The treatment containing TBHQ had a significantly higher PV than did the treatments containing any level of oat extract throughout the study. On day 6, the PV of the treatment containing TBHQ was much higher than any of the treatments with oat extract. The PV of the control was significantly higher than were PV of the rest of the treatments throughout the study.

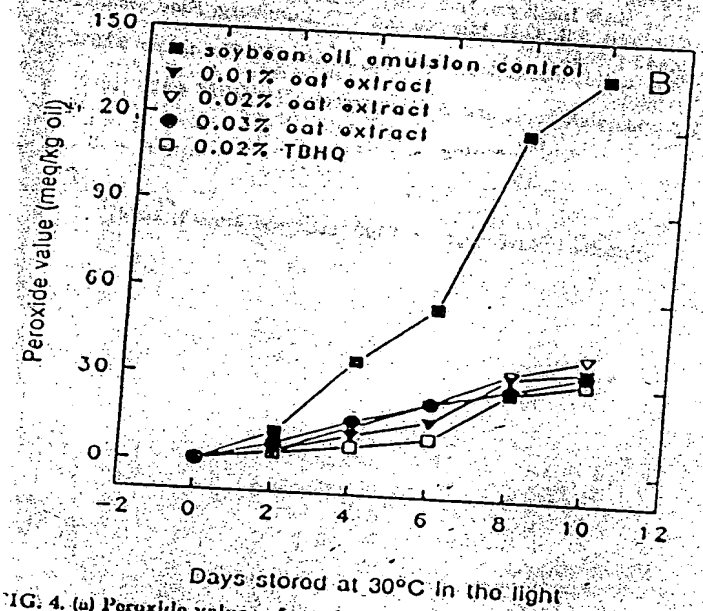
During storage of soybean oil emulsions at 30°C in the light (Fig. 4), the treatments containing added antioxidants had significantly lower PV than did the control oil. On day 2, no significant differences were found among the treatments containing different levels of oat extract and TBHQ. On days 4 and 6, the treatments containing different levels of oat extract were not significantly different from each other, but they had significantly higher PV than did the treatment containing TBHQ. On day 8, the treatments containing 0.03% oat extract and TBHQ, which were not significantly different from each other, had significantly lower PV than did the treatments containing 0.01 and 0.02% oat extract. Practically speaking, however, the differences among the antioxidant-treated emulsions probably were not important. On day 10, no significant differences were found among the treatments containing different levels of oat extract and TBHQ.

During 10 d of storage at 60°C in the dark (Fig. 5), the soybean oil treatments with all levels of oat extract and TBHQ had significantly lower PV than did the control. On day 2, treatments containing the different additives were not significantly different from each other, although, by day 4, the treatments containing any level of oat extract had significantly lower PV than did the treatment containing TBHQ. By day 6, the treatment containing 0.01% oat extract had a significantly higher PV than did the treatments containing the other two levels of oat extract, but all the treatments with oat extract had significantly lower PV than did the treatment containing TBHQ. On days 8 and 10, the treatment containing 0.03% oat extract had a lower PV than did the treatments containing the other two levels of oat extract, which were not significantly different from each other. Under conditions of 60°C storage in the dark, the oat antioxidants were far superior to TBHQ at reducing oxidation of the soybean oil emulsions.

In general, oat extract was more useful as an antioxidant in emulsions than was TBHQ, especially at 60°C storage in the dark. This effect may be because there are several components in oat extract giving a range of solubilities in different systems, thus allowing some compounds to be more soluble at the oil/water interphase in the oil and some more soluble in the water. In contrast, TBHQ, a single compound, was more soluble just in the oil phase. These results agree with those of Mushor (3),







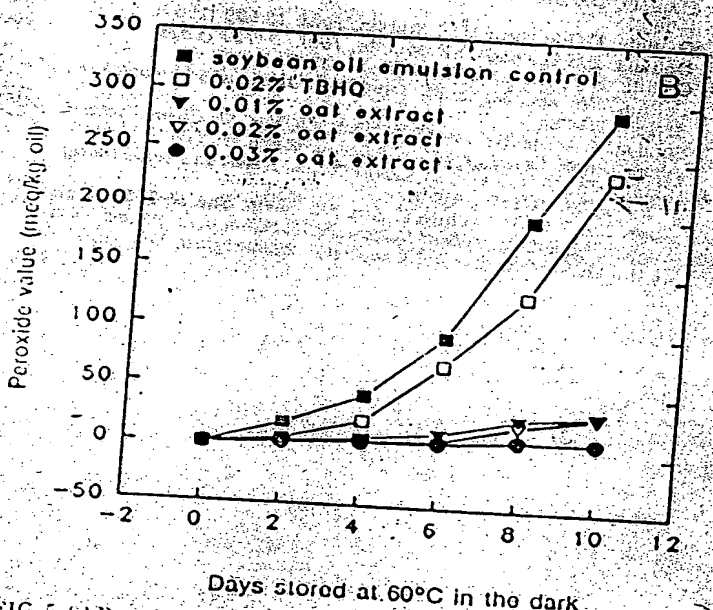


FIG. 4. (a) Peroxide values of cottonseed and (b) soybean oil emulsions stored at 30°C in the light. Abbreviation as in Figure 1.

FIG. 5. (a) Peroxide values of cottonseed and (b) soybean oil emulsions stored at 60°C in the dark. Abbreviation as in Figure 1.

ho reported that an aqueous extract of oat was markedly fective in protecting oil and fat emulsions. Chipault *et* . (33) also found that most of 32 spices they tested in ground form were more effective against oxygen absorpn in lard emulsions than in plain lard.

Quantity of extract needed. The amount of oat needed protect oil, when added at a level of 0.01% extract, sed on actual phenolic content, was 3.3 parts oat to 1 part oil: a great quantity of oat! Those figures are based on obtaining 30 mL of oat extract from 1 kg of oats, with the extract having a total phenolic content of 0.1% (wt/vol). Obviously, this amount of oat is too great to provide an economical source of natural antioxidants unless the antioxidant extraction is coupled with the production of other products from the same oats. For example, antioxidants may be obtained after the extraction of oil from

high-oil- containing oats. The extract from oat hulls, which contains phenolic antioxidants and Δ^5-avenasterol (14,34,35), may be another way to lower the cost of oat antioxidants. Oat hulls make up about 30% (wt/wt) of whole oats.

ACKNOWLEDGMENTS

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THE CHEMISTRY AND PHYSIOLOGICAL FUNCTIONS OF SESAME

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ABSTRACT

Some historical facts on and botanical descriptions of sesame are given. Some flavor studies of raw and roasted sesame seeds and oils are described. Composition and some usages are also briefly reported. Sesame has long been regarded in the Orient as a health food which increases energy and prevents aging. Sesame oil has been known empirically as a cooking oil which is highly resistant to oxidative deterioration in comparison with other edible oils. Until recently there were no scientific studies to elucidate these interesting aspects of sesame seed and oil, but the author and members of his group initiated studies on the chemical elucidation of antioxidative principles of sesame seed and oil, and extensively investigated the antiaging effect of sesame. Presence of various new antioxidative lignan phenol compounds in sesame seed and oil is described. Sesaminol has been identified as a new antioxidative principle in raw sesame salad oil. The mechanism of the superior antioxidative activity of roasted sesame oil is being elucidated and is consistent with the synergistic effect of the browning products with tocopherol, sesamol, and sesamin. Noticeable results concerning the antiaging effect of sesame have been shown in a series of animal experiments. The suppressive effect on senescence in mice by long-term feeding of sesame was demonstrated. Sesame lignans had a synergistic effect on vitamin E activities when added to tocopherols. The addition of sesame lignans, especially that of antioxidative lignan ses-

Table 1. Composition of Sesame and Main Food Materials (per 100 g)

	Sesame	Soybean	Rice		Wheat flour
			Unpolished	Polished	
Energy (calories)	578	471	351	356	368
Moisture (%)	4.7	12.5	15.5	15.5	14.0
Protein (g)	19.8	35.3	7.4	6.8	9.0
Fat (g)	51.9	19.0	3.0	1.3	1.8
Carbohydrate (g)	15.3	23.7	71.8	75.5	74.6
Fiber (g)	3.1	4.5	1.0	0.3	0.2
Ash (g)	5.2	5.0	1.3	0.6	0.4
Ca (mg)	1200	240	10	6	20
P (mg)	540	580	300	140	75
Fe (mg)	9.6	9.4	1.1	0.5	0.6
Na (mg)	2	1	2	2	2
K (mg)	400	1900	250	110	100
Vit. A (IU)	0	0	0	0	0
Carotene (μg)	17	12	0	0	0
B_1 (mg)	0.95	0.83	0.54	0.12	0.04
B_2 (mg)	0.25	0.30	0.05	0.03	0.04
Niacin (mg)	5.1	2.2	4.5	1.4	0.7
Vit. C	0	0	0	0	0

Fatty acids of the oil consist mainly of oleic and linoleic acids, with small amounts of palmitic and stearic acids but with only trace amounts of linolenic acid. A comparison of the fatty acids in sesame and of soybean and other oils is given in Table 3 (20).

Nutritionally, linoleic, linolenic, and arachidonic acids are considered as essential fatty acids (11), although arachidonic acid is assumed to be synthesized *in vivo* from linoleic acid. According to recent studies on prostaglandins, those produced from *n*-3 fatty acids (e.g., linolenic acid) are different from those derived from *n*-6 acids (e.g., linoleic acid) and have platelet anticoagulative action (21). In this connection it has been said that sesame oil with low *n*-3 fatty acid content is an inferior source of prostaglandin, but this would not place sesame oil much further behind other oils in nutritive value. If sesame oil is used in combination with soybean oil, as it is commonly used in preparing tempura in Japan, sesame oil markedly enhances the nutritive value of the lipid and increases the vitamin E activity.

Another important physiological action of unsaturated fatty acid is the suppression of plasma cholesterol levels. In one study on the effect of addition of

Table 2. Effe
tents of Minor

Number of sa
Oil (%)
Mean
Range
CV[b]
Sesamin (% in
Mean
Range
CV[b]
Sesamolin (%
Mean
Range
CV[b]
100 seed weig
Mean
Range
CV[b]
Hull[c] (%)
Mean
Range
CV[b]

[a]Mean value
nificantly at the
[b]Coefficient
[c]Ratio of the
[d]The conten
significantly on

oils to lard, the order o
ame oil > corn oil, th

Protein

Sesame contains appro
cording to Kinman (2).
Amino acid content va
is found between whit
values recommended b
Health Organization (F

cooked rice or bread, or mixed with vegetables as a dressing. It can also serve as a dip for boiled meat. Sesame powder was not available until freeze grinding was introduced.

"Goma-dofu" (sesame tofu curd) is made by solidifying a half-and-half mixture of sesame paste and arrowroot starch. It has a pleasant texture and is highly nutritious. In China, sesame jam, a mixture of sesame paste, fats, and sugar, is prepared in the form of a steamed flour dumpling. It is a very popular dish.

Black sesame seeds have been popular as food in Asian countries, partly because of a traditional belief that it prevents senility. How much truth there is in this belief is discussed later.

Oil meal, residue after oil is expelled, is highly valued as a food material with high nutritional value provided that the temperature of the expeller is not too high. Bread and meat loaf containing the meal have been developed (7–9). Use of meal is important because of sesame's high nutritional value of its proteins and lignans.

SESAME SEEDS: COMPOSITION AND NUTRITIONAL CHEMISTRY

As described above, sesame seeds vary considerably, depending on varieties, in size, color, and coat thickness. They differ in major and minor components.

Oil, protein, and carbohydrate are the major constituents of sesame. Those of a common brown sesame seed are shown in Table 1 (10). Though it may not be fully justified to compare sesame, a food only occasionally used, with food consumed daily, the nutritional value of sesame in comparison with soybean, rice, and others is discussed (11).

Oil

Sesame is a high-energy food containing approximately 50% oil, and cells of the cotyledon and the residual endosperm are filled with oil droplets. Many studies have shown the variation of the oil content by species and cultivation conditions: lowest is 34% to 35%, and highest is 63% to 64% (12–18). Recent data for 42 strains of sesame grown at two locations are given in Table 2 (19). The oil contents of the seed varied from 43.4% to 58.8%, the average being 52.7% and the standard deviation being 3.9%. The average oil content for the white-seeded strains was 55.0%; and for the black-seeded strains, 47.8%. The hulls of the black-seeded strains were thick; it was found that the oil contents varied inversely with the percentage of the hull, and that this could be used as a criterion to predict oil content. The correlation of oil content to lignan concentrations is discussed later in the sections on lignans.

Wheat flour
368
14.0
9.0
1.8
74.6
0.2
0.4
20
75
0.6
2
100
0
0
0.04
0.04
0.7
0

s, with small
s of linolenic
nd other oils

ered as essen-
synthesized *in*
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n-3 fatty acid
place sesame
s used in com-
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increases the

id is the sup-
f addition of

Table 2. Effect of Seed Color Types on Seed Oil Content and Contents of Minor Components in Oil of *Sesamum indicum* L.

	Seed color type		
	White	Brown	Black
Number of samples	15	12	11
Oil (%)			
Mean	55.0 a[a]	54.2 a	47.8 b
Range	51.8–58.8	50.5–56.5	43.4–51.1
CV[b]	3.7	3.4	5.9
Sesamin (% in oil)			
Mean	0.44 a	0.36 a[e]	0.24 b[e]
Range	0.12–0.61	0.11–0.61	0.07–0.40
CV[b]	36.7	38.8	39.0
Sesamolin (% in oil)			
Mean	0.25 a	0.30 a	0.27 a
Range	0.02–0.48	0.13–0.42	0.13–0.40
CV[b]	73.3	33.4	27.1
100 seed weight (mg)			
Mean	274.5 a	295.0 a	280.3 a
Range	228.8–390.9	218.7–346.3	232.5–351.9
CV[b]	12.8	18.8	12.9
Hull[c] (%)			
Mean	6.2 c	8.0 b	14.4 a
Range	3.5–8.3	6.1–9.5	6.7–23.2
CV[b]	25.5	12.4	39.3

[a] Mean values on each row followed by the same letter do not differ significantly at the 1% level.
[b] Coefficient of variation.
[c] Ratio of the hull to the whole seed by weight.
[e] The content of sesamin in oil in brown- and black-seeded strains differs significantly only at the 5% level.

oils to lard, the order of suppression of cholesterol level was soybean oil > sesame oil > corn oil, though this is still an unsolved issue (20).

Protein

Sesame contains approximately 20% protein [16.7–27.4%, average 22.3%, according to Kinman (2)]. The amino acid composition is shown in Table 4 (18). Amino acid content varies among species (15, 18), but no significant difference is found between white and black species (22). Compared with the standard values recommended by the Food and Agriculture Organization and the World Health Organization (FAO/WHO), sesame protein is slightly lower in lysine but

Table 3. Fatty Acid Composition of Vegetable Oils and Fat (g/100 g)

Fatty acid	Sesame oil	Soybean oil	Corn oil	Fat
10:0	–	–	–	0.10
12:0	0.29	0.10	–	0.91
14:0	0.14	0.16	–	3.71
16:0	9.4	10.7	10.7	2.48
18:0	4.76	3.87	1.74	18.7
20:0	0.58	0.22	0.29	–
14:1	–	–	–	1.55
16:1	0.30	0.29	0.14	4.73
16:2	–	–	–	0.88
18:1	39.1	22.8	24.6	36.0
18:2	40.0	50.8	57.4	3.65
18:3	0.46	6.76	0.82	0.56
20:1	0.21	–	–	–
22:1	0.38	–	–	–
Totals				
Saturated	15.2	15.0	12.2	48.2
Unsaturated (mono)	39.99	23.09	24.74	42.23
Unsaturated (poly)	40.46	57.51	58.22	5.09

richer in other amino acids, especially methionine, cystine, arginine, and leucine. Animal studies have shown that the simultaneous use of sesame protein and soybean protein, which is rich in lysine but low in methionine, produces good growth in rats (11, 23).

As already mentioned, sesame oil cake improves the nutritional quality of bread. It is highly valued as an additive to cattle feed, and comparative studies with sorghum, millet, and oil bean seed showed its value in improving the amino acid profile and protein utilization (24). However, the cake obtained from roasted sesame may contain heavily denaturized protein and was found unfit for food. Extraction of insoluble proteins and improvement of its nutritional quality by using bacterial enzymes are being investigated (25).

Carbohydrate

Carbohydrate content is about 18–20%, but there have not been many studies on the nutritional aspects. The presence of low amounts of glucose and fruc-

Table 4. protein)

Amino
Isoleucl
Leucine
Lysine
Methior
Cystine
Met +
Phenyla
Tyrosin
Phe +
Threoni
Tryptoj
Valine
Histidir
Arginin
Alanine
Aspartl
Glutam
Glycine
Proline
Serine

tose, and also an
furanosyl-α-D-gl
present. Most o
hydrates unaffe
of 11% has beer
reexamined, in
on various adult

Vitamins

Sesame contains
0.25 mg%, and

Minerals and Trace Constituents

Sesame seed is rich in mineral constituents, as shown in Tables 1 and 5. It is especially rich in calcium (120 mg/100 g) and iron (9.6 mg/100 g) as well as in phosphorous, potassium, magnesium, zinc, and selenium (32).

Calcium and iron, which are often deficient in modern diets, are found in high concentration in sesame. However, calcium is found to be contained mainly in the seed coat as an oxalate, Table 5. Recent investigation of the hot water and 0.1 M HCl extracts of the pulverized sesame by atomic absorption spectroscopy and ion chromatography showed that the contents of total and free oxalic acids are 1750 mg and 350 mg, respectively, and that of total calcium is 800 mg per 100 g seeds. Therefore, the nutritionally available calcium was estimated to be 165 mg, the difference between the content of calcium in calcium oxalate and the total calcium (33).

Selenium is also present in sesame. Selenium is a constituent of glutathine peroxidase, which is associated with the prevention of physiological peroxidation; but in excessive amounts, it has a negative effect. The human requirement is assumed to be 40–200 mg/day. One paper reported the content of 36.1 mg Se/g in isolated sesame protein (32). However, the Se content is related to the concentration in the soils.

A survey of the potentially harmful levels of Ni and other heavy metals in various vegetable oilseeds and oils showed that sesame seed was highest in Cu content (17.0 ppm) and contained Fe (80.7 ppm) and Ni (1.46 ppm). Cu (0.02 ppm) and Fe (0.14 ppm) were found in sesame oils, and Ni was not (34).

Lignans

Lignans, low molecular weight compounds produced by oxidative coupling of *p*-hydroxyphenylpropane, are a minor in amount but very important functional components of sesame. Sesame contains significant amounts of characteristic lignans, sesamin (a typical lignan with β–β linkage) and sesamolin (a compound with a phenylgroup with an acetal oxygen bridge), Figure 1 (35). Sesamin has been found in other plants also, but sesamolin seems to be characteristic of sesame. Commercial sesame seeds contain these lignans in fairly high levels as shown in Table 7.A (36). In this study, the lignan content of 14 varieties was determined. These varieties were grown in Japan and cultivated under the same conditions. Some difference was observed in the sesamolin content, but no apparent genetic and color differences were noted. Statistical analysis showed that the average ratio of sesamin to sesamolin in the black varieties was larger than in the white varieties at the 5% significance level.

Further investigation of the oil and lignan contents of sesamin and sesamolin in 42 strains of *Sesamum indicum* L. indicated that the percentage of sesamin

Figure 1. Lignans and antioxidants isolated from sesame seed and oil.

Table 7. Lignan C

Strain no.*	Ty
48	BC
611	BC
630	BA
638	3B
643	3B
785	3B
673	3B
675	3B
126	3B
201	3B
601	BA
631	3B
792	BA
801	BA
Mean	
SD	

B.

Strain no.	Ses
48	2
611	2
630	2
638	1
643	1
785	Tr
673	2
675	Tr
126	4
201	3
601	10
631	2
792	4
801	6
Mean	3
SD	2

*Strain and type: I
*Yellow: light yell

Table 7. Lignan Contents in Different Strains of Sesame Seed in Japan

A. *Sesamin and Sesamolin (mg/100 g oil)*

Strain no.[a]	Type[a]	Seed color[b]	Sesamin A	Sesamolin B	B/A
48	BON	White	821.3	441.2	0.537
611	BON	White	410.6	441.2	0.537
630	BAN	White	522.7	123.5	0.236
638	3BA	White	885.2	476.5	0.538
643	3BO	White	464.0	229.4	0.494
785	3BO	Yellow*	453.3	247.0	0.545
673	3BO	Violet**	464.0	317.6	0.684
675	3BO	Brown	528.0	264.6	0.501
126	3BA	Brown***	682.7	458.8	0.672
201	3BO	Black	502.5	441.2	0.878
601	BAN	Black	314.3	235.3	0.749
631	3BA	Black	362.7	229.4	0.632
792	BAN	Black	154.7	152.9	0.988
801	BAN	Black	293.3	294.0	1.002
Mean			490.6	300.4	
SD			198.6	113.6	

B. *Sesamol, P1, Sesamolinol, and Sesaminol (mg/100 g)*

Strain no.	Sesamol	P1 (a)	Sesamolinol (b)	Sesaminol (c)	Total (a) + (b) + (c)
48	2.0	1.6	1.0	1.4	4.0
611	2.5	1.3	1.0	1.0	3.3
630	2.5	2.3	0.9	0.3	3.5
638	ND	2.9	1.1	1.0	5.0
643	5.0	2.0	1.1	1.0	4.1
785	Trace	2.0	0.9	0.3	3.2
673	2.5	1.8	1.5	1.1	4.4
675	Trace	3.8	0.6	0.7	5.1
126	4.0	2.9	1.2	1.0	4.2
201	3.6	2.5	1.2	1.1	4.7
601	10.8	1.6	1.9	1.1	4.6
631	2.5	1.5	0.8	0.5	2.8
792	4.9	1.5	0.9	0.9	3.3
801	6.5	1.6	1.1	1.2	3.9
Mean	3.4	2.1	1.1	0.9	4.0
SD	2.9	0.7	0.3	0.3	0.7

[a] Strain and type: Ref. 1, p. 13.
[b] Yellow: light yellow; violet: light violet; brown: dark brown.

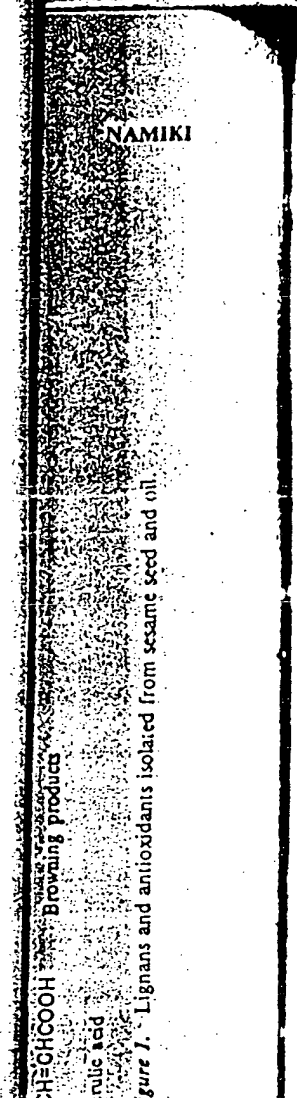

Figure 1. Lignans and antioxidants isolated from sesame seed and oil.

in the oil ranged from 0.07% to 0.61%, and that of sesamolin from 0.02% to 0.48%. There was a significant positive correlation between the oil content of the seed and the sesamin content of the oil ($r = -0.608$, significant at 1% level), while no correlation was found between the oil and sesamolin contents (19). The white- and black-seeded strains also differed significantly in sesamin content, but not in sesmolin content. The relationships between the ratio of sesamolin to sesamin and the oil content of *Sesamum* strains with different seed color types are shown in Figure 2. The four types are characterized as follows: White-seeded strain are of two types; Type A has a relatively high ratio of sesamolin to sesamin and a high oil content, and Type B has a markedly low ratio of sesamolin to sesamin and a high oil content. Brown-seeded strains form a cluster similar to that of white-seeded strains, Type A. Black-seeded strains have a high ratio of sesamolin to sesamin because of the low sesamin content and a low oil content. Yellow-seeded strains have a low ratio of sesamolin to sesamin and a high oil content (19).

Among the wild species recently studied by our group, an Indian variety had a marked lower sesamolin content (sesamin 256.1 mg/100 g and sesamolin 35.6 mg/100 g), and one from Borneo contained several times more of the amount of sesamin (1152.3 mg/100 g) and of sesamolin (1360.7 mg/100 g) than in other species (36).

The presence of the other lignans, sesangolin from a wild sesame species *S. angolense* (37), and 2-episesalatin from *S. alatum* (38), has been reported. Two new lignans, sesaminol and sesamolinol, with antioxidative properties, were isolated with pinoresinol by the author's group (39) and are discussed later. As

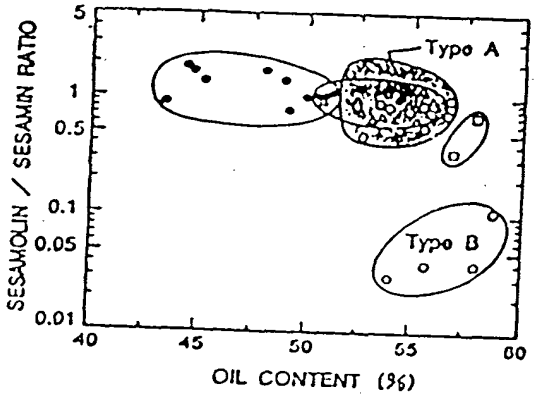

Figure 2. Relationship of the ratios of sesamolin content to sesamin content and to the oil content in different strains. ○, white-seeded strain; △, brown-seeded strain; ●, black-seeded strain; □, yellow-seeded strain.

shown in Table 7.B (36), the contents of these antioxidative lignans (free phenol form) in sesame seed were very small as compared with sesamin.

Because antioxidative activity was developed by treatment of defatted sesame cake with β-glucosidase, Osawa, Katsuzaki et al. (40–42) looked for water-soluble and potential antioxidative lignans in sesame seeds. These new diglucosides and one new triglucoside of pinoresinol (Fig. 1) and three new glucosides of sesaminol were isolated by high-pressure liquid chromatography (HPLC), and structures of their aglycone and sugar moieties were determined by gas chromatography–mass spectroscopy (GC–MS), and liquid chromatography–mass spectroscopy (LC–MS) analyses as described later (43).

Other Minor Components

Sesame lipids contain traces of triterpenes and steroids, though in lesser amounts than present in corn. Kamal-Eldin et al. (44) compared various chromatographic methods for separation and quantification of sesame lignans, tocopherols, and sterols in *Sesamum indium* L. and three wild species. From the unsaponifiables of the oils, campesterol, stigmasterol, sitosterol, and Δ⁵ avenasterol as the major desmethyl sterols, and obtusifoliol, gramisterol, cycloeucalenol, and citrostandienol as monomethyl sterols were determined (44).

Contents of phytic acid and oxalic acid are higher than in other oil sources (45). Phytic acid that is combined with Zn is regarded to cause Zn deficiency. Ca, Mg, and metal phytates serve as antioxidant. Oxalic acid is said to be present as the calcium salt.

The oil from roasted sesame oil sometimes forms precipitates during the clarification process. Analysis of the precipitates indicated that the main constituents are dicarboxylic acids, mostly octacosanedioic acid and four others which are seldom found in vegetable oils (46). Sterylglycosides (isofuco-, campe-, stigma-, and sito-steryl) were also identified as minor constituents. The dicarboxylic acids and sterylglycosides in the roasted sesame oil were present at 0.5% and 700 ppm, respectively, and 0–1500 ppm and 1–300 ppm in commercial sesame oil, respectively (47).

It has also been reported that a hair root culture of sesame (*Sesamum indicum* L.), which had been established by transformation of the mother plant with *Agronobacterium rhizogenes* ATCC15834, produced a significant amount of the antimicrobial compound 2-isopropenylnaphthazarin-2,3 epoxide (over 50-fold that found in the mother plant), as well as two new anthraquinone derivatives (48).

Recent phytochemical investigations of *Sesamum* species have shown the presence of a iridoid glycoside (49), phenylethanoid glycoside (50), and triterpenoids (51). Suzuki et al. (52), by using various chromatographic methods followed by nuclear magnetic resonance (NMR) and MS analyses, isolated and

identified, from water extracts of whole plants of *Sesamum indicum*, eight phenylethanoid glycosides including two new ones and three triglycosides which had the identical sugar sequence.

OIL CHEMISTRY AND PRODUCTION

Because of its high oil content in sesame, sesame oil could readily be produced by simple, primitive techniques of using pressurized steam or by expressing oil from roasted sesame seeds. In modern industry, the use of expellers is almost universal although small hydraulic presses are sometimes used. A modern expeller can produce about 40–50 t/day. A small hydraulic press produces only about 50 kg/day, but it is said to produce a higher-quality oil (1).

Two different types of sesame oil are produced: one is from roasted seed; and the other, from seed cooked with steam (Fig. 3). The former (roasted sesame oil) is classified according to roasting temperature and time of roast (e.g., 140°–150°, 160°–180°, and about 200°C; from several minutes to 10–30 min). The expelled oil is simply filtered without further purification. Its color ranges from light to dark brown and it has a characteristic roasted flavor, the intensity depending on the roasting conditions.

The latter (unroasted or raw sesame oil, also called sesame salad oil) is further processed by degumming, alkali washing, water washing, decolorization,

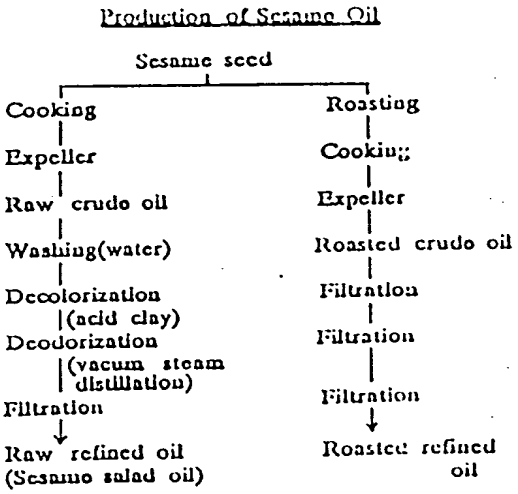

Figure 3. Production of sesame oil.

browning products were adsorped on XAD-7 column, and oil which was eluted was both light in color and low in antioxidative activity. The adsorbed material was eluted from the XAD-7 column with ethyl acetate and then chromatographed on a silica gel column, and the browning products (BP), separated from tocopherol, sesamol, and sesamin, were obtained by elution with methanol. The browning products thus obtained were further separated with HPLC to yield several fractions. The antioxidative activity tests using the weighing method indicate that the browning products alone showed weak antioxidative activities, but the addition of BP to the following systems brought about significantly increased antioxidative activities in each case: γ-tocopherol (0.05%), γ-tocopherol + sesamol (0.01%), γ-tocopherol + sesamin (1.0%), and γ-tocopherol + sesamol + sesamin. The synergistic effect was especially eminent in the combination of γ-tocopherol + BP. The system of γ-tocopherol + BP + sesamol + sesamin showed so much antioxidative activity that the superior antioxidative activity of the roasted sesame oil could only be explained by the synergistic effect of these four components. The effective synergistic components in the browning products and the relationship between the effectiveness and the color remains yet to be determined (97).

In another study of the antioxidative activity, the water, methanol, and ether extracts of roasted sesame seed on the stability of fish oil (sardine oil) was examined. The results showed that the water and methanol extracts were not effective, but the ether extract was effective in minimizing rancidity (99).

Other Antioxidants in Sesame Seeds and Oils

Water-Soluble Lignan Antioxidants in Sesame Seeds

Antioxidants of lignan phenols, such as sesaminol, sesamolinol, and pinoresinol, were isolated from sesame seed with organic solvents. They were not very water soluble, and the amounts isolated from the seeds were small (39).

Fukuda et al. (100) found a marked increase in the antioxidative activity of sesame seeds and sprouts extracted with methanol during germination. Concentrations of sesamin, sesamolin, and γ-tocopherol decreased with sprout growth, while the concentration of phenolic substances increased markedly, suggesting the increase of free phenolic substances released by hydrolysis of their glycosides during germination.

Utilization of sesame oil cake is important in the development of new food and feed products that have improved nutritional and physiological qualities.

Investigations (40–42) have been initiated to look for hydrophilic lignan antioxidants in seeds and their potential as water-soluble antioxidants. Ground, defatted sesame was extracted with 80% ethanol, followed by chromatographic separation along with the testing of antioxidative activity of the fractions. Three

new glycosides of pinoresinol were isolated and identified: pinoresinol 4'-*O*-β-D-glucopyranosyl(1-6)-β-D-glucopyranoside, pinoresinol-4'-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranoside, and pinoresinol 4'-*O*-β-D-glucopyranosyl(1-2)-*O*-[β-D-glucopyranoside(1-6)]-β-D-glucoside, and one known pinoresinol di-*O*-β-D-glucopyranoside. The three new products showed antioxidative activities in the linoleic acid autoxidation system as well as in the *t*-butyl hydroperoxide induced peroxidation of erythrocyte ghost membrane system (105).

These investigators have also identified three new glucosides of sesaminol in sesame seeds: sesaminol 2'-*O*-β-D-glucopyranoside, sesaminol 2'-*O*-β-D-glucopyranosyl-(1—2)-β-D-glucopyranoside, and sesaminol 2'-*O*-β-D-glucopyranosyl-(1—2)-*O*-[β-D-glucopyranosyl(1—6)]-β-D-glucopyranoside. These are considered as potential antioxidants to develop the activity by action of β-glucosidase.

Antioxidative Substances in Black Seed

According to tradition, black sesame is more effective as a health food than white or brown sesame seeds, but no chemical or physiological studies have been conducted to confirm this belief.

No significant differences in amino acid composition were found between black and white sesame seeds (101). Tashiro et al. (19) reported that significant differences exist in their oil and lignan contents. The black-seeded strains had a higher percentage of hull and a lower percentage of oil than did the white- and brown-seeded strains.

Because of the traditional belief that black sesame has a superior antiaging effect, Fukuda et al. (102) examined the antioxidative activities of the extracts from black seeds and their fractions, and they compared similar materials obtained from white seeds. They studied black and white seeds of 14 domestic strains. The antioxidative activities of 80% ethanol extracts from crushed black and white seeds were not significantly different although the black seeds seemed to have more activity than the white. The water extracts of seed coats from 10 different strains were examined (4 black, 2 brown, and 4 white). All of the black and brown strains showed strong antioxidant activity, but only 1 in 4 of the white seeds showed any activity.

The black pigment in the coat (hull) was more soluble in distilled water than in ethanol or chloroform and appeared to be a tannin. This black pigment, when chromatographed, showed several peaks, and two of these exhibited marked antioxidative properties (102).

Antioxidative Substances in Cell Culture of Sesame

Mimura et al. (103) produced antioxidative products by cell culture of sesame. Callus cells were induced from stems, leaves, and root cells from sprouts from sesame plants, with use of a modified Murashige-Skoog medium. Cells were successfully cultured from sesame callus cells in a similar liquid medium. It was

noted that growth rate was higher with higher yields at relatively higher incubation temperatures (about 35°C) than at the usual plant cell culture temperatures (25°–27°C). Analyses of a methanol extract from cultured sesame cells showed the presence of sesamin and sesamolin but no antioxidative compounds such as sesamol, γ-tocopherol, or ascorbic acid. However, the extract had a strong antioxidative activity in the linoleic acid autoxidation system as well as in the peroxide-induced erythrocyte ghost cell membrane system. Antioxidative components in the cells were isolated and identified as new caffeic acid glucosides. These antioxidative products were as active as BHA. Utilization of these antioxidative products obtained from cultured sesame cells in antioxidant preparations (skin lotions) to protect against light-catalyzed oxidation has been proposed (104).

Evaluation of Sesame Antioxidants in a Model Biological System

Much attention is now focused on damage to membranes, nucleic acids, and proteins by active oxygen species produced by reduction of molecular oxygen and by lipid peroxidation. This damage causes circulatory ailments, senility, mutations, and cancer. Metaloenzymes, such as superoxide dismutase (SOD) and catalase, are known to suppress formation of active oxygens and their reactions, as well as vitamins E, C, and A present in foods. However, except for these vitamins, little is known about the effect of antioxidative food constituents. The presence of sesaminol and other powerful antioxidants in sesame seeds and its oil has been described. Not only are they active as preservatives, but also, they may have a role in physiological suppression of lipid peroxidation.

Prior to the animal experiments which are mentioned later, the antioxidative activities of sesaminol, sesamol, and other antioxidants were examined in the following lipid peroxidation systems as models of *in vivo* peroxidation: (*a*) on the peroxidation of ghost membranes of rabbit erythrocyte induced by *t*-butylhydroperoxide and analysis by thiobarbituric acid (TBA), and (*b*) on the peroxidation of rat liver microsome (105). The latter involves two modes of peroxidation: the ADP-Fe^{3+}/NADPH system in which NADPH cytochrome P450 reductase is involved, and hydrogen is directly withdrawn from unsaturated fatty acids by peripheryl ion, and the ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH system in which peroxidation is initiated by the formation of activated oxygen by the action of Fe^{3+} on fatty acid hydroperoxides. The results showed that sesame lignan phenols have suppressive activity to lipid peroxidation equal to or stronger than tocopherol in systems (*a*) and (*b*). Table 13 shows the results in system (*b*) (105).

In other studies the suppressive activity of sesaminol was observed on lipid peroxidation induced by *t*-butylhydroperoxide in cultured human diploid fibroblasts. Sesaminol was as strongly suppressive as tocopherol in mutagenicity of *E. coli* WP2s induced by peroxidation of membrane lipid of erythrocytes (105).

Table 13. Relative Antioxidative Activity of Lignanphenols in Sesame Using Rat Liver Microsome

	ADP-Fe^{3+}/NADPH	ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH
Control	100.0	100.0
P1	14.9	13.2
Sesamolinol	4.6	6.3
Sesaminol	8.6	10.3
Pinoresinol	17.2	14.4
Sesamol	24.1	19.0
α-Tocopherol	9.2	19.0

ANTIOXIDATIVE ACTIVITY OF SESAME *IN VIVO*

As mentioned earlier, an ancient Chinese natural history book reported that habitual ingestion of sesame prevents problems associated with aging. Based on our chemical knowledge of the highly antioxidative properties of sesame seeds and oil, we examined the antioxidative and antiaging effects of sesame seeds, oil, and antioxidative lignans *in vivo*.

Effect of Sesame on Senescence of Mice

In experiments studying the effect of sesame on senescence, we used senescence-accelerated mice (SAM) developed by Takeda et al. at Kyoto University (106). Their original strain is an AKR strain of mice. The R series are senescence-resistant mice with a normal aging process. The P series are a specific type of SAM. P/1 mice were used in our experiments. The SAM showed characteristic symptoms of senescence in behavior and appearance, that is, loss of activity, lack of hair glossiness, skin coarseness, and most typically periophthalmic lesions. Some pathological changes were also observed, such as inflammed tissues and amyloidosis. The degree of senescence in mice was evaluated according to a grading system developed by Takeda et al. (106).

Changes in SAM senescence scores during long-term feeding with either a standard diet or a diet containing 20% black sesame powder are shown in Figure 7 (107). With the standard diet, every measure of senescence increased after about 4–5 months, but in the case of the sesame diet, increases were slow and suppressed, especially with respect to periophthalmic lesions, hair glossiness, and skin coarseness. The overall appearance was quite different between these two groups. After 7 months, the levels of lipofuscin, a pigment related to aging, were slightly suppressed in SAM liver and testes. The SOD activity in the liver was clearly in-

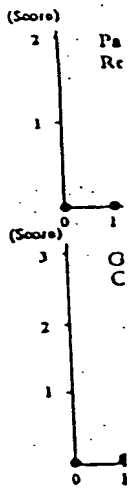

Figure 7.

me Using Rat Liver

A-Fe^{3+}/NADPH
0.0
0.2
0.3
0.3
4.4
9.0
9.0

book reported that with aging. Based on ties of sesame seeds ts of sesame seeds,

we used senescence- to University (106). senescence-resistant c type of SAM. P/1 acteristic symptoms activity, lack of hair almic lesions. Some ed tissues and amy- cording to a grading

ng with either a stan- e shown in Figure 7 increased after about slow and suppressed, ness, and skin coarse- ese two groups. After g, were slightly sup- liver was clearly in-

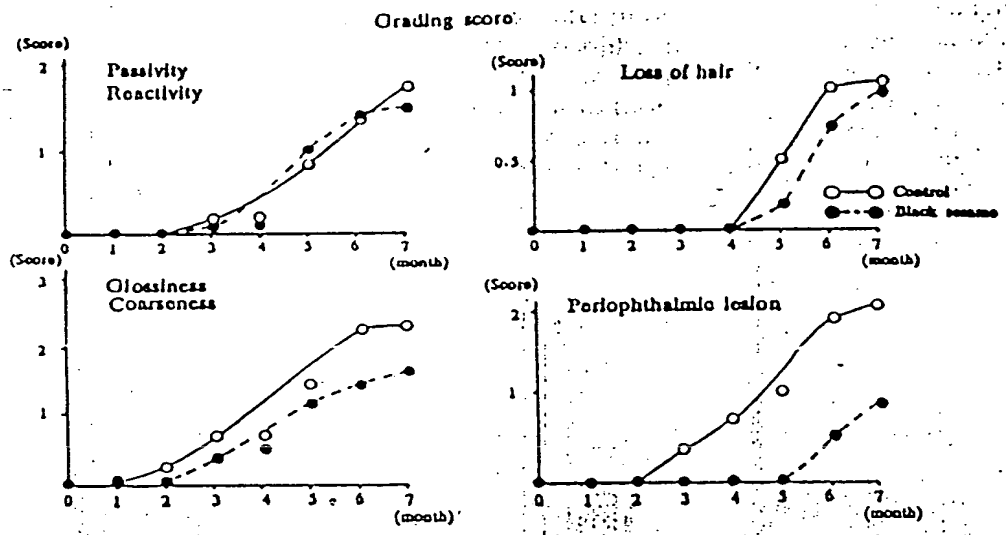

Figure 7. Effect of sesame on senescence score of SAM. SAM groups were fed for 7 months, with the standard diet (AIN-76) (control) and a diet containing 20% black sesame powder (•—•). Protein and fat contents in these diets were adjusted to the same level. Each score has five grades, from 0 to 4 (108).

creased in the SAM fed black sesame, though the levels of plasma thiobarbituric acid-reactive substance (TBARS) did not differ significantly.

The suppressive effect on SAM senescence was also observed with the addition of sesaminol, the new antioxidant isolated from unroasted sesame oil, to a 50% vitamin E-deficient diet (107). In the effect of sesaminol on lipid peroxidation of SAM, no significant suppression of TBARS values in liver or kidney was observed when sesaminol was added to a diet containing enough vitamin E, but in the vitamin E-deficient diet, this ingredient apparently suppressed any increase in TBARS. The suppressive effect of sesaminol on *in vivo* lipid peroxidation was also observed in the increase of liver TBARS levels when CCl_4 was administered to rats (107, 108).

Synergistic Effect of Sesame and Its Lignans on γ-Tocopherol

The fact that sesame and sesaminol suppressed senescence of SAM as well as lipid peroxidation *in vivo* led us to ask whether one principal component is responsible for the suppressing effect or whether it is the result of the combined activity of several components.

Vitamin E is recognized as a food component that may have an antiaging effect (109), because of its antioxidative activity. In most foods, α-tocopherol is the major isomer in vitamin E, and γ-tocopherol is only 6–16% as active as α-tocopherol (110), even though it has stronger antioxidative activity than α-tocopherol in *in vitro* (111). The tocopherol of sesame seed is predominantly γ-tocopherol, with only trace amounts of α-tocopherol (28), which means that sesame should be poor in vitamin E activity.

In order to clarify this, the vitamin E effect of sesame was compared with that of α-tocopherol and γ-tocopherol (112). In this study, the control rats were fed for 8 weeks a vitamin E-free diet. The test groups were fed the same vitamin E-free diet plus α-tocopherol, γ-tocopherol, sesame, and sesaminol. Lipid peroxidation (plasma and liver TBARS values), oxidative hemolysis, and plasma pyruvate kinase activity were determined as indices of vitamin E status. As shown in Figure 8, the peroxide concentrations of plasma and liver were greater in the vitamin E-free control than in the + α-tocopherol group. The + γ-tocopherol group showed significantly higher peroxide concentrations than did the + α-tocopherol group, and the + sesame group had concentrations as low as those of the α-tocopherol group. Pyruvate kinase activities were similar to the peroxide concentrations. The most significant difference was observed in red cell hemo-

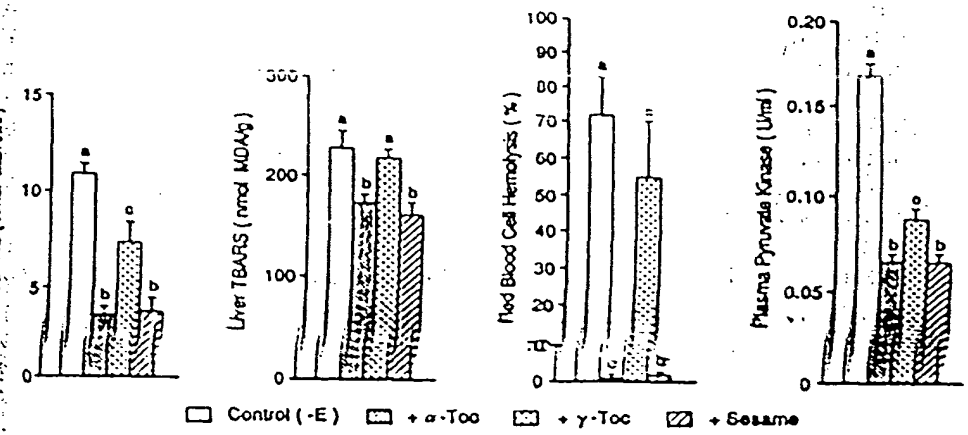

Figure 8. Effect of sesame seed on lipid peroxides in plasma and liver, red blood cell hemolysis, and plasma pyruvate kinase activity. The content of α- or γ-tocopherol in the α- or γ-tocopherol-containing diet was adjusted to equal that of γ-tocopherol in the sesame seed diet (51.7 mg/kg). Lipid peroxide concentrations were measured by the thiobarbituric acid method, and hemolysis test was performed using dialuric acid. Values are means ± SEM, $n = 6$. Values with different superscripts are significantly different, $p < 0.05$. MDA = malondialdehyde; TBARS = thiobarbituric acid-reactive substance.

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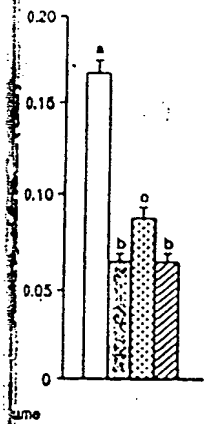

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lysis. The + γ-tocopherol group only weakly suppressed the increase of the hemolysis, whereas in the sesame fed groups, hemolysis was almost completely suppressed as in the group which was fed α-tocopherol, despite the fact that sesame contains only a negligible amount of α-tocopherol.

In these experiments, we found that plasma and liver concentrations of α-tocopherol in rats were only high in the + α-tocopherol fed group; the concentrations of γ-tocopherol were substantial only in the sesame seed-fed group, and were very low in the + γ-tocopherol group, although the sesame diet and + γ-tocopherol diet contained equal amounts of γ-tocopherol. These results suggested the presence of some components in sesame that cause an increase of γ-tocopherol concentrations in plasma and liver, presumably resulting in the prevention of increase in TBARS and other indices caused by a vitamin E-free diet (112).

Experiments were therefore conducted on the vitamin E-free diets and similar diets including + γ-tocopherol and sesame lignans, that is, sesaminol or sesamin. As shown in Figure 9, the combination of γ-tocopherol + lignans, especially sesaminol, prevented an increase in the indices of vitamin E deficiency, results similar to those obtained on an E-free diet by the addition of α-tocopherol or sesame. In addition, γ-tocopherol concentrations in plasma and liver were also observed as in the case of + sesame group. The antioxidant sesaminol is

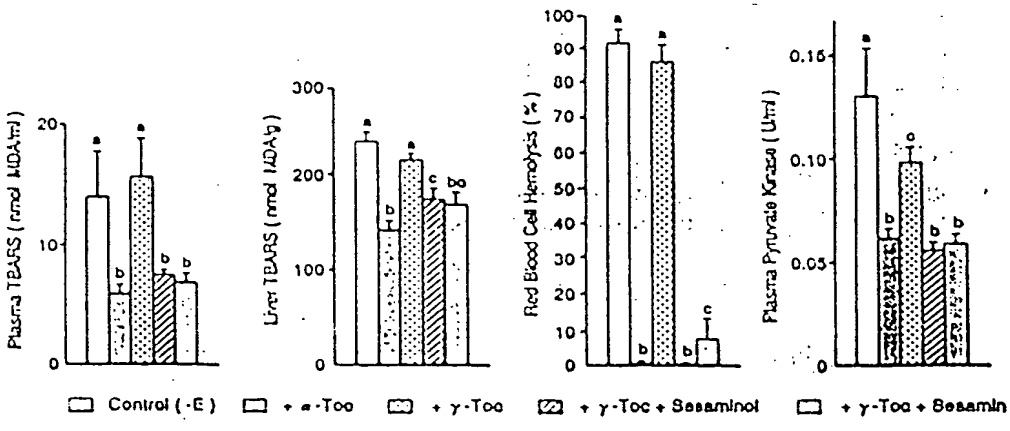


Figure 9. Synergistic effect of sesame lignans with γ-tocopherol on lipid peroxides in plasma and liver, red blood cell hemolysis, and plasma pyruvate kinase activity. 50 mg α- or γ-tocopherol/kg was added to the tocopherol-containing diets and 2 g sesaminol or sesamin/kg was added to the sesame lignan-containing diets. Values are means ± SEM, $n = 6$. Values with different superscripts are significantly different, $p < 0.05$. MDA = malondialdehyde; TBARS = thiobarbituric acid-reactive substance.

superior to non-antioxidant lignan sesamin in this regard. It may be that the synergistic effect of sesame lignans may result in enhancing the vitamin E activity of γ-tocopherol and as a result prevent lipid peroxidation *in vivo*, and may also contribute to the preventive effects of sesame on lipid peroxidation *in vivo* and thereafter on senescence in SAM.

The mechanism of this interesting synergistic effect of lignans is not yet clear but may be due to their action on the metabolism of γ-tocopherol. The weak vitamin E activity of γ-tocopherol can be assumed to be caused by weaker bonding activity to transporting proteins in blood and cellular membranes in the liver than that for α-tocopherol.

Synergistic Effect of Sesame Lignans on α-Tocopherol

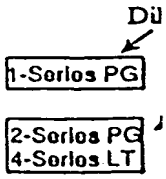
Studies on the synergistic effect of sesame lignans on vitamin E activity of γ-tocopherol led to finding a marked enhancing effect of sesame lignans on vitamin E activity of α-tocopherol. In experiments similar to those described above for determining vitamin E activity, it was demonstrated that the rat group fed a reduced amount of α-tocopherol (1/5 to the standard + E control group) showed a marked increase in the lipid peroxidation *in vivo*, but that increase was completely suppressed by the addition of 5% of sesame in the diet. Addition of sesaminol or sesamin instead of sesame also produced a marked enhancement of vitamin E activity in the same manner, although sesaminol, a strong antioxidant, was more effective than sesamin. In these experiments it was observed that the addition of sesame as well as its lignans resulted in high tocopherol concentrations in blood and liver. This might produce high vitamin E activity and prevent an increase in lipid peroxidation. The details of this enhancing mechanism are not yet understood (113).

The novel enhancing effect of sesame lignans, especially antioxidative lignan, on the vitamin E activity of tocopherols brings new problems to the evaluation of various foods containing sesame seeds and oil. For example, if we have a soybean paste containing sesame seed paste, or if we eat some fish or vegetables fried with sesame oil, the enhancing effect of the sesame lignans on vitamin E activity of the tocopherols in these foods will give a higher vitamin E value because of the synergistic effect of the lignans. This enhancing effect may necessitate assigning a higher value of vitamin E activity assigned to these foods based simply on tocopherol concentrations.

OTHER PHYSIOLOGICAL EFFECTS OF SESAME LIGNANS

Effect of Sesame Lignans on Linoleic Acid Metabolism and Eicosanoid Production

Highly unsaturated fatty acids, such as arachidonic acid, dihomo-γ-linolenic acid, and eicosapentanoic acid (EPA) have important biological functions, in



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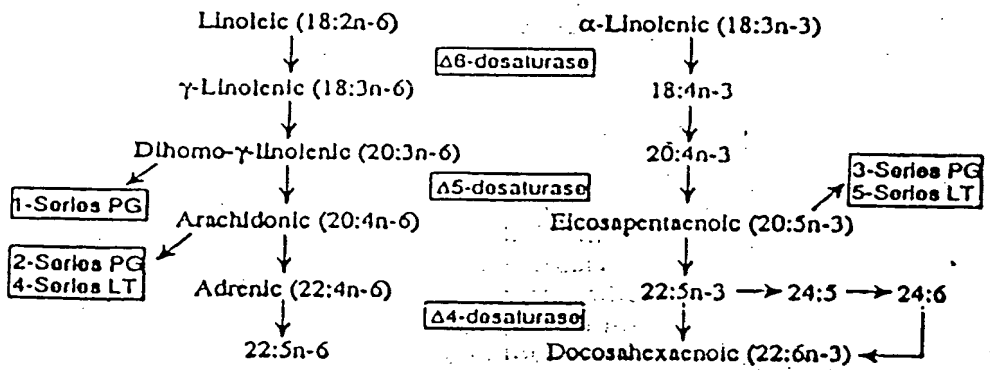

Figure 10. Desaturation and elongation of polyunsaturated fatty acids, and the production of eicosanoids.

particular as precursors of prostaglandins, Figure 10. To develop large-scale production of these unsaturated fatty acids using a microbiological procedures, the Yamada and Shimizu groups (114, 115) screened various microorganisms and found that fungal mycelia are rich sources of these fatty acids. There is an especially high yield of arachidonic acid with *Moritierella alpina* 1S-4 (114) and of EPA with *M. alpina* 20-17 (115). They also found that incubation with sesame oil specifically increased the dihomo-γ-linolenic acid (20:3*n*-6) content and decreased in the arachidonic acid content (116). This interesting effect of sesame oil was demonstrated to be caused by the specific inhibiting activity of sesamin and other lignans present in sesame on the Δ5-desaturase in polyunsaturated fatty acid biosynthesis in microorganisms, as shown in Table 14 (117).

This fact is of particular interest to those studying the metabolism of polyunsaturated fatty acids in animals because different eicosanoids affect distinct biological functions. Sugano et al. (118) demonstrated that sesamin interferes with Δ5-desaturation even in liver microsomes, which results in considerable modification of the fatty acid profile of liver phospholipids. The proportion of *n*-6 eicosatrienoic acids increased when animals were fed sesamin. Sesamin also tended to decrease the plasma concentration of PGE_2 (119). However, no influence on the aortic production of prostacyclin nor on the production of thromboxane A_2 by platelets was observed (118).

The specific inhibition of Δ5-desaturase and chain elongation of C_{18} fatty acids by sesamin, especially in *n*-6 polyunsaturated fatty acid biosynthesis, was also observed in rat primary cultured hepatocytes (120).

Hypocholesterolemic Activity of Sesamin and Mechanism of Action

Sugano et al. (119) also reported on the hypocholesterolic activity of sesamin. As shown in Table 15, sesamin reduced blood cholesterol levels of rats which

Table 14. Specific Inhibition of Fungal and Rat Liver Δ5 Desaturases by Sesamin-Related Compounds[a]

Compound added	Desaturase activity (pmol/min/mg protein)						
	M. alpina				Rat liver		
	Δ9	Δ12	Δ6	Δ5	Δ9	Δ6	Δ5
Sesamin	10.5	9.27	19.1	2.80	34.9	16.4	71.8
Episesamin	10.3	8.40	18.2	5.20	36.1	17.5	92.1
Sesaminol	10.1	8.37	18.4	4.67	36.0	15.4	87.9
Sesamolin	9.93	8.77	17.7	3.97	37.7	18.6	86.6
Sesamol	9.86	8.10	17.2	16.8	34.3	14.7	114

[a] Desaturase activities were measured as described in Ref. 117 except that each of the indicated compounds was added and incubations with the liver microsomes were carried out for 30 min. All the compounds were present at 28 or 85 μM in the reaction mixtures with *M. alpina* extract or rat liver microsomes, respectively. Values are means of three independent assays (standard deviation, within ± 7%).

were fed a purified diet or commercial chow irrespective of dietary cholesterol. Sesamin also reduced the concentration of liver cholesterol, especially in diets containing cholesterol. Further studies showed that sesamin increased fecal excretion of neutral steroids but did not show any effect on excretion of acidic steroids and bile acids. This phenomenon may be due to the inhibition of intestinal absorption of cholesterol by sesamin. In addition, it was also found that

Table 15. Effect of Sesamin on the Concentration of Serum and Liver Cholesterol in Rats[a]

Group	Serum cholesterol (mg/dL)[b]	Liver cholesterol (mg/dL)[b]
Experiment with purified diet		
Cholesterol-free diet	108 ± 4 a	2.54 ± 0.13 a
Cholesterol-free diet + 0.5% sesamin	110 ± 5 a	1.95 ± 0.06 b
Cholesterol diet	136 ± 8 b	20.8 ± 2.2 c
Cholesterol diet + 0.5% sesamin	102 ± 5 a	9.13 ± 1.02 d
Experiment with commercial chow		
Cholesterol-free diet	69.1 ± 5.2 a	2.86 ± 0.19 a
Cholesterol-free + 0.5% sesamin	55.5 ± 3.0 b	1.82 ± 0.04 b

[a] Values are means ± SE of 6 to 8 rats.
[b] In each experiment, values with different letters are significantly different at $p < 0.05$.

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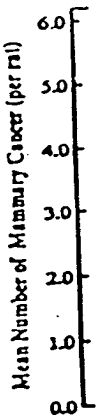

Figure 11. El
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sesamin reduced the activity of hepatic 3-hydroxy-3-methylglutaryl CoA reductase, the key enzyme in the cholesterol synthesis. However, it did not influence bile acid synthesis as estimated from the cholesterol 7α-hydroxylase in liver microsome (118). These results indicate a unique function of sesamin on cholesterol dynamics because there is no other compound that simultaneously inhibits both cholesterol absorption and synthesis. This means that sesamin can serve as an efficient natural hypocholesterolemic agent, and clinical trials are currently underway. Significant reduction in lymphatic cholesterol and fatty acids in rats fed a 24% sesame oil diet compared with coconut oil and corn oil has also been reported (121). In this report a probable effect of the different sterols in these oils has been noted. However, based on the above experiments, it may be due to the hypocholesterolemic effect of sesame lignans.

Preventive Effect of Sesamin on Chemically Induced Mammary Cancer

The liver activating and antioxidant activities of sesamin led to the belief that sesamin might be a potential anticarcinogen. Sugano et al. (122) examined the effect of sesamin on 7,12-dimethylbenz-(α)-anthracene-induced rat mammary cancers, and found that sesamin at the dietary level of 0.2% considerably reduced the cumulative number and the mean number of mammary cancers as compared to controls, Figure 11 (122).

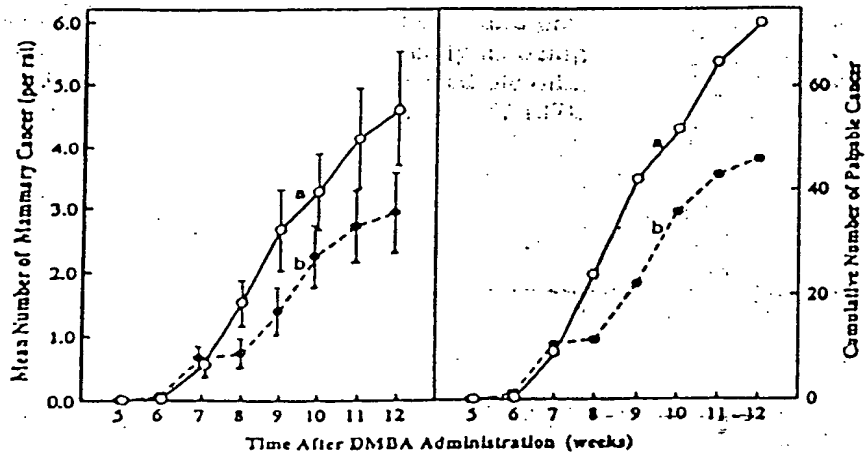

Figure 11. Effect of sesamin on chemically induced mammary cancer in rats. Values are means ± SE of 15 rats per group. Lines bearing different letters are significantly different at $p < 0.05$.

Table 16. Effect of Sesamin on Serum Chemistries and Liver Histology in Mice Receiving Continuous Inhalation of Ethanol[a]

Group	Serum chemistries: Total bilirubin (mg/dL)	Serum chemistries: GOT activity (IU/L)	Serum chemistries: GPT activity (IU/L)	Fat droplets in hepatocytes[b]
Control	0.47 ± 0.15	150 ± 76	26.1 ± 7.8	1.0
Ethanol	1.61 ± 1.32[†]	312 ± 204[†]	39.6 ± 31.9	3.5
Ethanol + sesamin	0.41 ± 0.04[*]	81.6 ± 15.4[**†]	18.3 ± 1.6[*]	1.8

[a] Values are means ± SE of 7 mice.

[b] Livers with no lipid droplets and a large number of lipid particles in the lobules were given a score of 1 and 4, respectively.

[*] $p < 0.05$, [**] $p < 0.01$ vs. ethanol group; [†] $p < 0.05$ vs. control group.

Effect of Sesamin on Liver Functions

Rats fed a diet containing sesamin at levels above 0.5% frequency showed enlargement of the liver, although no abnormal tissue changes were observed (118). The activity of serum GOT and GPT remained unchanged, but some enzyme activities seemed to be enhanced by sesamin. In fact, as shown in Table 16, studies in which mice fed a diet containing sesamin were exposed to high concentration of carbon tetrachloride or ethanol showed an improvement in liver function as estimated from aminotransferase activity, and concentrations of total cholesterol, triglycerides, and total bilirubin in blood (123). Subsequent studies showed that rats previously given sesamin reduced their plasma alcohols levels more rapidly than the control rats. This interesting effect of sesamin was further examined in human trials. A group of male adults moderately deficient in aldehyde dehydrogenase were given sesamin (100 mg/day for 7 days) or a placebo. They were then given a drink of whiskey equivalent to 60 mL of alcohol. The skin temperature was monitored by an infrared camera. The temperature of all the subjects' faces rose rapidly, reached a peak, and then fell gradually. There was a significant difference in the rate of reduction of blood ethanol level between groups receiving sesamin or a placebo. Based on these observations, droplets containing sesamin and α-tocopherol are being commercially distributed in Japan.

CONCLUDING REMARKS

Sesame has long been regarded as a health food which increases energy and prevents aging. Sesame oil as a cooking oil has been known empirically as being highly resistant to oxidative deterioration in comparison with other edible oils. To explain the antioxidative stability of sesame oil, Olcott et al., in 1941 (87),

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and Budowski et al., in 1950 (88), cited the presence of sesamol and its effective antioxidative activity, but the reasons for the superior antioxidative activity remained unclear. Chemical studies on sesame lignans as a characteristic minor component were developed by Budowski in 1951 (56), Beroza in 1956 (94), and others; but little was known about the physiological activities of sesame lignans except for the synergistic effect of sesamin on pyrethrine insecticides and some other physicopathological effects (56).

The author and members of his group initiated studies on the chemical elucidation of antioxidative principles of sesame seed and oil, and extensively investigated the physiological explanation of the antiaging effect of sesame in relation to the strong antioxidative activity. Presence of various antioxidative components involving new antioxidative lignan phenol compounds in sesame seed and oil was elucidated by Osawa, Fukuda, and others in 1985 (91). Sesaminol was identified as a new antioxidative principle in raw sesame salad oil, with its interesting formation via an intermolecular rearrangement from sesamolin in the decolorization process. A better understanding of the mechanism of the superior antioxidative activity of roasted sesame oil is emerging that is consistent with the synergistic effect of the browning products with tocopherol, sesamol, and sesamin.

Noticeable results concerning the antiaging effect of sesame have been shown in a series of animal experiments conducted by Yamashita and Namiki in 1990 (107). With senescence accelerated mice (SAM), long-term feeding of sesame as well as its antioxidative lignans demonstrated the effectiveness of these compounds in suppressing senescence. The studies were developed to look for the novel synergistic effect of sesame lignans to tocopherols in their vitamin E activities. The addition of sesame lignans, especially that of antioxidative lignan sesaminol in the diets of rats, markedly enhanced vitamin E activity of γ-tocopherol to the same level of α-tocopherol, and also significantly enhanced the vitamin E activity of α-tocopherol. These effects were accompanied by a marked increase in the concentration of these tocopherols in blood and liver. The enhancement of vitamin E activity by lignans is very important in evaluating vitamin E activity as well as the antiaging effect of various foods.

Other interesting physiological effects of sesame lignans have been developed from the studies on microbial production of polyunsaturated fatty acids conducted by Yamada, Shimizu et al. in 1988 (115). It was found that sesame lignans specifically inhibit $\Delta 5$ desaturase in the process of formation of arachidonic acid from linoleic acid. This was also demonstrated in animal experiments by Sugano et al., in 1990 (118). Various interesting physiological activities of sesame lignans in animal and human tests were shown, such as hypocholesterolemic activity, suppressive activity of chemically induced cancer, and enhancing effect on various liver activities involving detoxication of carbon tetrachloride and ethanol.

These recent developments in chemical and physiological studies on sesame seed and oil seem to partly unveil the mystery surrounding sesame, though there remain many interesting physiological activities in various aspects of advanced nutritional and physiological sciences which need to be clarified.

Future studies should focus on the physiological activities of lignans. Biochemical investigations should be on:

1. The metabolism of lignans in the liver and the interactioins with some enzymes, proteins, and cell membrane constituents
2. The mechanism of the suppression of oxidative damage *in vivo* in combination with tocopherols
3. The effect of antioxidative lignans on various diseases assumed to be caused by oxidative stress
4. The correlation between chemical structure and physiological activities of lignans involving various other compounds, etc.

Studies are needed on lignans from plant physiological aspects:

1. On biosynthesis of lignans, especially of sesamolin, which has a characteristic acetal structure and a potential component to produce sesaminol
2. On gene technological investigations to improve production of lignans, not only in quantity but also in quality, to increase sesamolin and sesaminol contents
3. On cell culture techniques for production of lignans and other useful metabolites

Recent studies on sesame demonstrate that sesame, though only a minor constituent of daily diets, plays an important role in developing the potential powers of other food constituents and markedly raises food quality not only in traditionally evaluated aroma and taste but also in nutritional and physiological aspects. The addition of sesame in any form to soybean foods, such as fermented soybean paste (miso), enhances vitamin E activity and makes the essential amino acids nutritionally more available.

Much attention has been focused on the effect of the daily diet on health, especially on circulatory disorders, carcinogenesis, and senility. Because of advances in this new field of elucidation of unknown physiological functionalities of various foods, the Ministry of Health and Welfare of Japan recently approved some foods to be designated as "food for specified health use." The U.S. National Cancer Institute has also initiated a food program designed to show the functionalities of vegetable foods in the prevention of cancer. In view of these developments, sesame seed and oil should be considered as one of the most valuable foods for good health and quality of life.

An important consideration at present is how to broaden and increase the utilization of this excellent health food on a worldwide scale. Annual production

of sesame is 2,500,000 tons (2,430,000 tons in 1992, FAO); this is far smaller than other vegetable oil crops such as soybean oil, rapeseed oil, and others, although it has been increasing gradually. About two thirds of the sesame crop was used in oil production and the other one third was used in various foods. At present, although sesame oil is widely used as cooking oil in the Orient, most usage of roasted oil is as a very precious seasoning oil. In Japan, however, roasted and unroasted sesame oils are used in frying and as a salad oil. Usage of sesame oil as frying and salad oils in the USA and Europe is also very small compared to other vegetable oils. The use of sesame oil for frying and as a salad oil, as well as an additive to other vegetable oils, should be encouraged.

Consumption of sesame seed as food is closely related to food habits and the art of cooking. Throughout the world, sesame is mostly utilized as a whole seed in condiments or as additives on breads, biscuits, and other cereal products. The seed has a fairly hard coat and is not easily digested and is therefore undesirable for general consumption. For sesame to be more digestible, it should be used as ground sesame or a paste. For more popular use of sesame, research in food science and technology should be initiated to improve its flavor and texture. Perhaps the variety of ways sesame is utilized in Japanese and Chinese cooking can be extended to other cuisines.

In order to increase consumption of sesame, the economics of the production of sesame must be considered. The market price of sesame oil is considerably higher than those of other vegetable oils, probably because of low production of sesame seed. Research efforts should be made to improve varieties of sesame plants by genetics and by increasing yields by improved cultivation methods. The nutritional quality can be improved by increasing the concentration of the beneficial components.

ACKNOWLEDGMENTS

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...ution of Lignan Analogues to Antioxidative Activity of ...d Unroasted Sesame Seed Oil

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...mpound, P3, having strong antioxidative ...found to be formed in high concentration ...industrial bleaching process of unroasted ...d oil. P3 (named sesaminol) was identical to a ...tituent previously isolated from acetone ...sesame seed. It was shown that sesamolin in ...d sesame oil is the source of sesaminol, and ...n of sesaminol was confirmed by the model ...with corn oil to which sesamolin had been ...minol was not so greatly removed by the ...ion process that follows bleaching as was ...It was shown to be at a concentration of ca. ...in commercial refined unroasted seed oil. ...tive activity of sesaminol was roughly ...ce of sesamol and γ-tocopherol by the ...method. Therefore, it seems that the ...activity of refined unroasted seed oil is ...ted to sesaminol.

...of sesame oils, from unroasted seed and ...are widely used. Both of these oils have ...to be resistant to oxidation. The oxidation ...ed oil, commonly used throughout the world ...times, has remained obscure, in spite of chem...tion of the oil by Budowski et al. (1–4) and ...al. (5). They reported that sesamol was a ...oxidant and was produced from sesamolin ...aching process with acid clay, but was nearly ...removed from the oil by the subsequent ...process. We reported previously (6) that ...from both unroasted and roasted seeds ...able amounts of γ-tocopherol, but in spite of ...the amount of tocopherol during refining ...the refined unroasted seed oil had stronger ...ive activity than the crude oil. However, the ...ld not be shown.

...ent work deals with investigation of the ...ive principles in refined unroasted seed oil.

...S AND METHODS

...Sesame oil at various stages of processing ...kali-treated, washed, bleached and deodor...ized clay were donated by Takemoto Oil Co. ...ori, Japan. Corn oil was a gift from Oji Corn ...Tokyo, Japan, and γ-tocopherol was given by ...Tokyo, Japan. Sesamol (reagent grade) was ...from Sigma, St. Louis, Missouri. Sesamolin and ...e purified as reported previously (6) and their ...confirmed by mass spectrometry and proton ...tic resonance (¹H-NMR). Linoleic acid was ...distillation. Silica gel (BW 820-H from Fuji... ...used for column chromatography.

...formance liquid chromatography (HPLC). ...filtered oil sample was injected onto a ...ODS column, for analyses of γ-tocopherol, sesamin, sesamolin and sesamol. The eluting solvent was methanol for γ-tocopherol and 70% methanol for others. The mixture of sesamin and episesamin and that of P3 and its epimers were separated using Develosil SI 60-5, with 8:2 and 7:3 (v/v) *n*-hexane/ethyl acetate, respectivly, as solvents.

Thin layer chromatography (TLC). TLC was performed on a Merck 60F$_{254}$ Silica-plate.

Antioxidative activity. The thiocyanate method was employed to measure the degree of oxidation of linoleic acid in the samples as previously described (7).

Instruments. Those used were a Hitachi 200-10 Spectrometer for UV, JEOL JNM-FX-200 for NMR, JEOL JNM-D-100 for mass spectra, JMS-OISG for high resolution mass spectra and JASCO DIP-4 for specific rotation.

RESULTS

Isolation of antioxidants and some lignans from refined unroasted seed oil. Comparison of HPLC patterns at 290 nm between crude and refined unroasted seed oil was made to investigate chemical changes in sesamin, sesamolin, sesamol, etc. As shown in Figure 1, larg...

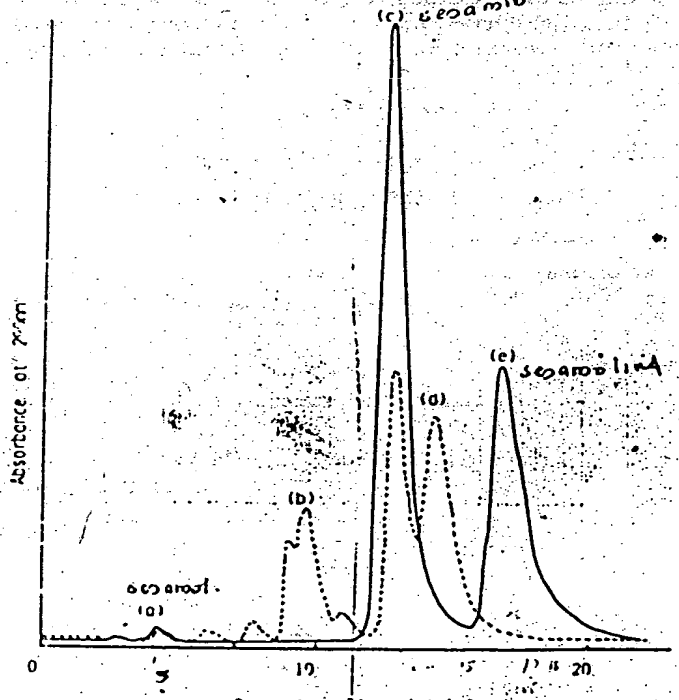

FIG 1. Comparison of HPLC patterns in crude and refined oils from unroasted sesame. Column, Develosil 10-ODS; eluent, MeOH-H$_2$O (7:3); flow rate, 3 ml/min; detector, UV 290 nm. ———, Crude oil; - - - -, refined oil. (a), Sesamol; (b), unknown; (c), (+)sesamin; (d), unknown; (e), sesamolin.

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differences were observed. From comparison of retention times with authentic sesamin, sesamolin and sesamol, it was concluded that peak (a) was sesamol, peak (c) was sesamin and peak (e) was sesamolin. It was also shown that some refining process eliminated peak (a) and gave rise to several new peaks, including (b) and (d). Peak (b) gave a reddish-purple color with ferric chloride on the TLC plate and was considered to be a phenolic compound.

The procedure adopted for isolation and purification of the antioxidants from refined oil is outlined in Figure 2. The fraction of 9-1 (*n*-hexane-EtOAc) silica gel chromatography was oily eluents including a small amount of γ-tocopherol. The amount of soluble components in the more polar fractions beyond 7-3 (*n*-hexane-EtOAc) silica gel chromatography was very small; hence, in this report the isolation of those fractions was not carried out. F1 was crystallized from methanol and identified as (+)episesamin from the data of mass fragment ions, ^{1}H-NMR, optical rotation and mp in comparison with the literature (8,9). The retention time of F1 in HPLC agreed with that of peak (d) in Figure 1. F2 was identified as (+)sesamin by comparing with authentic (+)sesamin and agreed with that of peak (c) in Figure 1. F3 showed identical molecular formula and ^{1}H-NMR chemical shifts of the sesamin analogue P3 ($C_{20}H_{18}O_7$) isolated from sesame seeds (F3 was found to be identical to P3). F4 and F5 showed mol wt and fragmentation peaks identical with those of P3, but showed differences in H-2 and H-6 signals in the NMR spectra and in TLC Rf values ($CHCl_3$-Et_2O, 9:1). Hence F4 and F5 are assumed to be epimerica isomers of P3. The fact that episesamin was found in refined unroasted seed oil also supports P3 epimerization. F6 was identified as sesamol dimer upon compar... the mol wt and ^{1}H-NMR spectrum report... literature (10). The confirmative studies of P3 epimer are under way.

Identification of P3. P3, presently ide... "Sesaminol," has the composition $C_{20}H_{18}O_7$ 370.3840, C 64.86%, H 4.90%, found 37... 64.90%, H 4.94%) and showed mp 130–131 C, ... C (c = 1.0, $CHCl_3$), absorption maxa at 238 and ... ($\log_\epsilon$ = 3.99 and 4.17) in the UV spectrum and ... ions, 370 (M⁺100%), 353 (22.6), 203 (43), 186 (4... (41.4), 135 (54.3). Though sesaminol seems ... sesamolin-type lignan from the molecular form... ^{1}H-NMR spectrum of sesaminol was very ... that of (+)sesamin. The ^{1}H-NMR spectrum of ... and (+)sesamin is listed in Table 1 (11). The ... (+)sesamin is apparently symmetrical in the ... the ^{1}H-NMR signals for H-1/H-5, H-2/H-6 ... methylenedioxy signals (5.92, s), but in the ... spectrum of sesaminol, the signals for H-1/... H-2/H-6 were almost identical with those of ... min, but each of the methylenedioxy protons ... as singlets at 5.90 (2H) and 5.97 (2H). The ... region of sesaminol showed, in addition to th... seen in the (+)sesamin spectrum (6.83, m, 6H) ... 1,3,4-trisubstituted ring, two singlets at 6.43 ... supporting the fine coupling due to para-rela... This 2',5'-placement, two singlets of methyl ... protons, production of monoacetyl ether of ... (M⁺412) on acetylation with acetic anhyd... pyrizine, and positive reaction with $FeCl_3$ ... confirmed the structure of sesaminol as II in ... The position of the aromatic-OH was also co... X-ray analysis of benzoate of sesaminol ep... stereochemistry of sesaminol and its epimers ... be published elsewhere.

Analysis of lignans and tocopherol in unroa... oil during processing. Refining process... unroasted seed oil are as follows: alkali ... washing, bleaching with acid clay and deod... The amounts of the compounds (sesamin, ep...

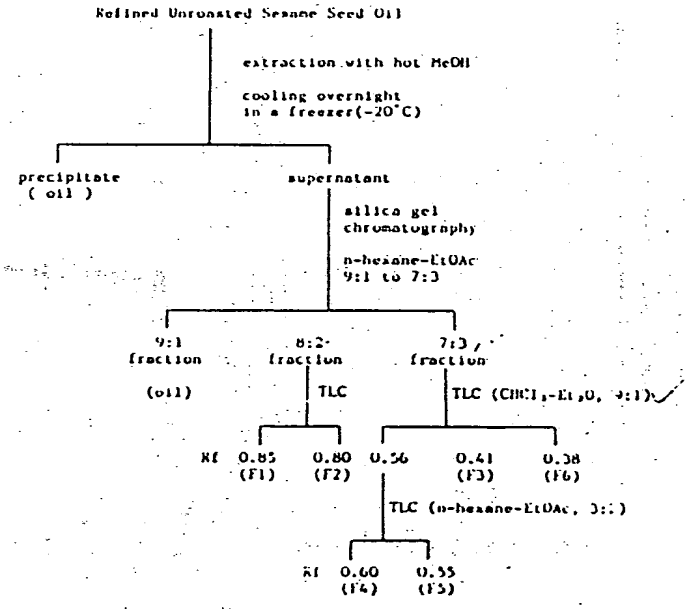


FIG. 2. Scheme for isolation of antioxidants from refined unroasted sesame seed oil.

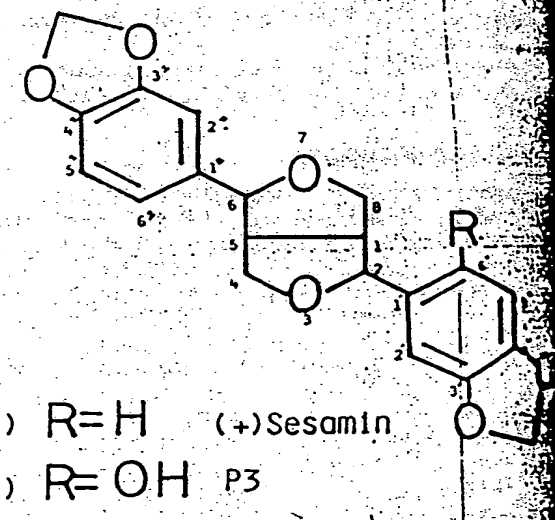


FIG. 3. Chemical structure of P3 and (+)sesamin.

TABLE 2

Amount of Lignans and Tocopherol in Unroasted Sesame Seed Oil During Refining Process (mg/100 g Oil)

Oil no.[a]	(+)Sesamin	(+)Episesamin	Sesamolin	Sesamol (its dimer)	Sesaminol (P3)	Episesaminol	γ-Tocopherol
1	813.3	0	510.0	4.3 (0)	0	0	33.5
2	730.6	0	458.0	2.5 (0)	0	0	23.4
3	677.8	0	424.8	0.7 (0)	0	0	22.6
4	375.5	277.6	0	46.3 (trace)	33.9	48.0	21.8
5	258.3	192.6	0	1.7 (trace)	28.4	34.3	18.4

[a]1, crude; 2, alkali-refined; 3, washed with warm water; 4, bleached; 5, deodorized. Amounts of antioxidants, sesamin and episesamin were analyzed by HPLC as described in the text.

TABLE 3

Amount of Sesaminol and Tocopherol in Different Commercial Sesame Oils (mg/100 g Oil)

Commercial sesame oil	Sesaminol	Epi-sesaminol	Total sesaminol	γ-Tocopherol
A	61.2	81.6	142.8	26.6
B	58.2	76.0	134.2	29.3
C	52.2	69.6	121.8	26.2
D	17.9	23.9	41.8	23.5
E	52.2	69.6	121.8	n.d.
F	6.6	8.8	15.8	n.d.

Amount of antioxidants was analyzed by HPLC as described in the text.

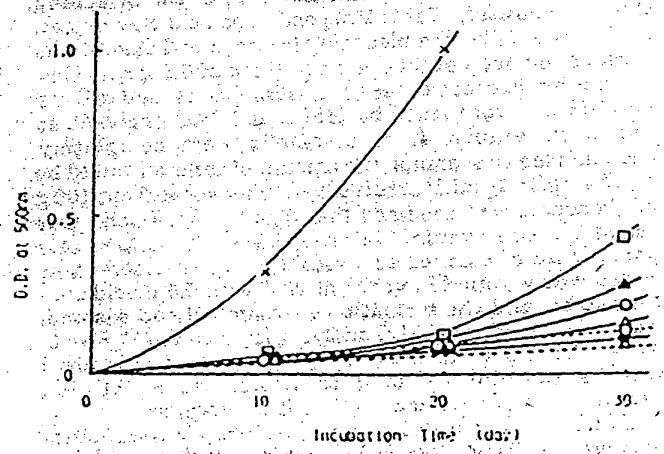


FIG. 6. Antioxidative assay of lignans in refined unroasted seed oil by the thiocyanate method. Amount of samples in the incubation mixtures (10 ml of 0.13 ml linoleic acid in 99% EtOH, 10 ml of 0.1 M phosphate buffer, pH 7.0, volume adjusted to 25 ml by H_2O). ×——×, Control; □——□, sesamol dimer (0.2 mg); ▲——▲, sesaminol (0.2 mg); ●——●, γ-tocopherol (0.2 mg); ○——○, sesaminol (0.2 mg); ○- - -○, sesaminol (0.5 mg); △——△, episesaminol (0.2 mg); △- - -△, episesaminol (0.5 mg).

sesamol dimer and γ-tocopherol, using linole... substrate by the thiocyanate method (7), are... Figure 6. The antioxidative activities of ses... episesaminols were roughly equal to ses... γ-tocopherol. It is clear than sesaminol-... sesaminols are the dominant antioxidant in ... due to their potency and higher concentra... γ-tocopherol. Sesaminol was also highly hea... because a relatively large amount of residual ... remained after heating of the refined oil at 18... hr.

DISCUSSION

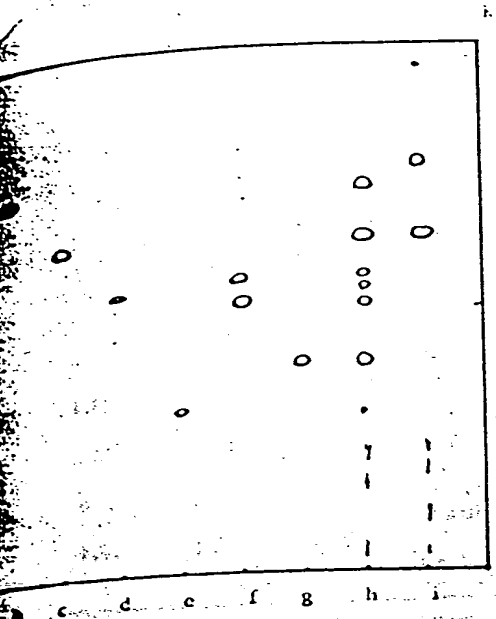
The increase in sesamol content during the ... process of the oil from unroasted sesame oil ... noted earlier by Honig (12), Budowski (13) and ... (14). A more quantitative and detailed pictu... antioxidant distribution was obtained in the ... work by the use of HPLC. The results newly ... show epimerization of (+)sesamin and c... conversion of sesamolin to sesaminol (a pre... transformation) and its epimers.

The recognition of sesaminol (and its epim... dominant antioxidant in refined oil appears ... considerable significance. Sesaminol, found in ... quantities in sesame seed and as its glycoside (n... of any taste, odor or color, has higher heat stab... might be considered one of the most important ... antioxidants for food. The antioxidative act... sesaminol in biological systems is also an in... topic related to the aging process, and is curren... study.

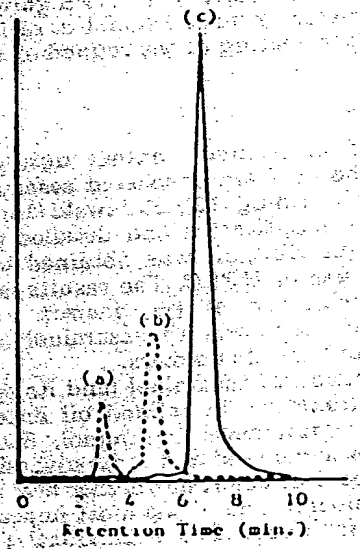
The conversion of sesamolin to sesaminol ... systems seems to be an acid-catalyzed r... involving scission and transformation of a C-Ob... also of interest as a chemical reaction, the d... which will be reported elsewhere.

ACKNOWLEDGMENTS

K. Tsuji, W.D. Crow and Ramarathanam Narasimh... valuable discussions.



...of methanol extract of oil (oil no. 3 in Table 2) ...acid clay. Plate, Merck Silica plate 60F$_{254}$; solvent, ... detector, UV lamp. a, Sesamolin; b, (+)epi... d, sesamol; e, sesamol dimer; f, episesa... MeOH extract from oil no. 3 add with acid ... in vacuo in 90 C water bath; i, MeOH extract ... heating, in vacuo in 90 C water bath.



...patterns of corn oil added with sesamolin and acid ... Develsil 10-ODS; eluent, MeOH-H$_2$O (8:2); flow ... ——: 2 ml corn oil + 5 mg sesamolin; heating 1 hr, ... water bath; – – – –; 2 ml corn oil + 5 mg sesamolin ... heating 1 hr, in vacuo in 90 C water bath. (a), Sesamol; ... sesamolin.

TABLE 1

^{1}H-NMR Data of Sesaminol (P3) and (+)Sesamin

Proton no.	Sesaminol (P3)	(+)Sesamin (11)
H-1/5	3.14 (2H,m)	2.88 (2H,m)
H-2/6	4.76 (2H,d, J=3.8)	4.75 (2H,d, J=4.0)
H-4a/8a	3.86 (2H,m)	3.74 (2H,d,d, J=4.0 and8.5)
H-4e/8e	4.36, 4.14 (2H,m)	4.10 (2H,d,d, J=6.0 and 8.5)
—OCH$_2$O—	5.90, 5.97 (4H,s)	5.92 (4H,s)
H-2'/5'	6.46, 6.53 (2H,s)	(Ar-H)
H-2"/5"/6"	6.80, 6.86 (3H,m)	6.83 (6H,m)
Ar-OH	7.6 (1H,s)	

δ Values, internal standard TMS, solutions in CDCl$_3$, 200 MHz spectra, J in Hz.

sesamolin, sesamol, sesamol dimer, sesaminol, its epimers and γ- tocopherol) in the oils at each step were determined by HPLC analysis. The results shown in Table 2 revealed that significant chemical changes took place mainly at the bleaching stage using acid clay. These were epimerization of sesamin (41%); disappearance of sesamolin; and production of sesamol, sesaminol, its epimers and a minor amount of sesamol dimer. That sesamol was produced from sesamolin during the bleaching step and was removed in the next deodorizing step has been reported by Budowski et al. (1-4), but the production of sesaminol after the bleaching step has not been reported before. Noteworthy is the fact that the significant amounts of sesaminol and its epimers produced were not as greatly decreased by the deodorization step as was sesamol.

The sesaminol and γ-tocopherol content in refined oil from three different sources is shown in Table 3. Sesaminol content varies from one product to anoth r, probably because of the difference in bleaching procedures. Of six samples, two from the same sourc contained no detectable amount of tocopherol.

Epimerization of sesamin and formation of sesaminol and its epimers. To investigate whether the chemical changes, namely, epimorization of (+)sesamin and production of sesamol and sesaminol, in the bleaching stage as shown in Table 2 depend upon acid clay or not, 2 ml of the oil before bleaching (oil no. 3 in Table 2) was warmed in vacuo at 90 C with addition of 0.5 g acid clay for one hr. Formation of (+)episesamin, sesaminol and sesamol was confirmed by HPLC and TLC analyses as shown in Figure 4. If sesamolin were completely decomposed to sesamol, the amount of sesamol would be ca. 154 mg/100 g oil. But actually sesamol was 46.3 mg/100 g oil; hence, it was assumed that a part of sesamolin was converted to sesaminol or its epimers. To confirm this point, 2 ml of corn oil to which 5 mg of sesamolin was added was warmed in vacuo at 90 C with 0.5 g acid clay for one hr, and the formation of sesaminol and sesamol were confirmed by HPLC analysis, as shown in Figure 5. These results confirmed the production of sesaminol from sesamolin by acid catalysis. More detailed examination of chemical aspects is in progress.

Comparison of antioxidative activities of antioxidants present in refined oil from unroasted seed. The results of comparative study of sesaminol, episesaminol, sesamol,

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ANNEXURE VI

Table 1. Comparison of free radical scavenging Activity

Sl.No	Sample	Conc. Of Antioxidant	Free radical Scavenging Effect after 30 min.
1	BHT	20 μ M	81.61
2	TBHQ	20 μ M	98.88
3	Catechin	20 μ M	98.51
4	Tannic Acid	20 μ M	98.88
5	Sesame cake extract with MeOH	1.925 mg/ml	98.43

Table 2. Radical quenching activity of antioxidants

Sample	EC_{50}	Antiradical power* (ARP)
Sesamol	75	13×10^{-3}
α-tocopherol	200	5×10^{-3}
Ascorbic acid	125	8×10^{-3}
BHT	300	3.3×10^{-3}
TBHQ	60	16×10^{-3}
Sesame cake extract(crude,methanolic	154×10^{-5}	0.648×10^{-5}
Sesame cake extract (purified)	6.4×10^{-5}	15×10^{-5}
Sesame seed extract	30×10^{-3}	3.33×10^{-5}

*Antiradical Power(ARP) = 1 / EC_{50}

As a result of the purification steps, the antiradical power has improved nearly 24 times as evident from values of crude and purified extracts respectively.

ANNEXURE VI

Table 3: Results of crude extraction studies of sesame seed/cake.

Sample	Extract weight (%)	Antioxidant lignans in ppm (ie.mg. per Kg.)			
		Sesamol	Sesamin	Sesamolin	Total
1.Sesame cake extracted with Methanol	20.2	2359 ~~477~~	4431 ~~895~~	936 ~~189~~	8285 ~~1561~~
2.Sesame cake extracted with Acetone	20.0	590 118	1661 332	--	2251 450
3.Sesame cake extracted with Ethanol	14.0	569 80	2608 365	892 125	4089 570
4.Sesame cake extracted with Ethyl acetate	12.4	926 116	5730 720	2120 266	8776 1102
5.Sesame cake extracted with Isopropanol	1.5	67.3	394	22	484
6.Sesame cake extracted with Hexane(ie.cakeoil	4.9	---	3220 157	trace	3220 157
7.Sesame seed extract with Methanol	28.5	3830 483	3998 510	2057 262	9885 1261
8.Purified extract from cake as per our process	5.0	22676 1108	1,05738 5168	12,500 611	1,40,914 6887
9.Sesame seed extract purified as per our process.	7.2	16733 2351	3951 555	2233 314	22917 3220

* Conditions of extraction: Soxhlet extraction by respective solvents for 16 hours in taking 10 g sample under same conditions. The extract weight is expressed as % of raw material weight. The antioxidant (lignan) content is expressed as 'parts per million' ie. milligrams of lignans present in 1 kg. of the extract. Alternately, the concentartion of lignan as milligrams present in 1 Kg. of the raw material is also calculated and given in blue ink.

The results clearly show that by our process, there is enrichment of antioxidant compounds by 4.5 times at least on raw material weight basis itself.

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ANNEXURE VII

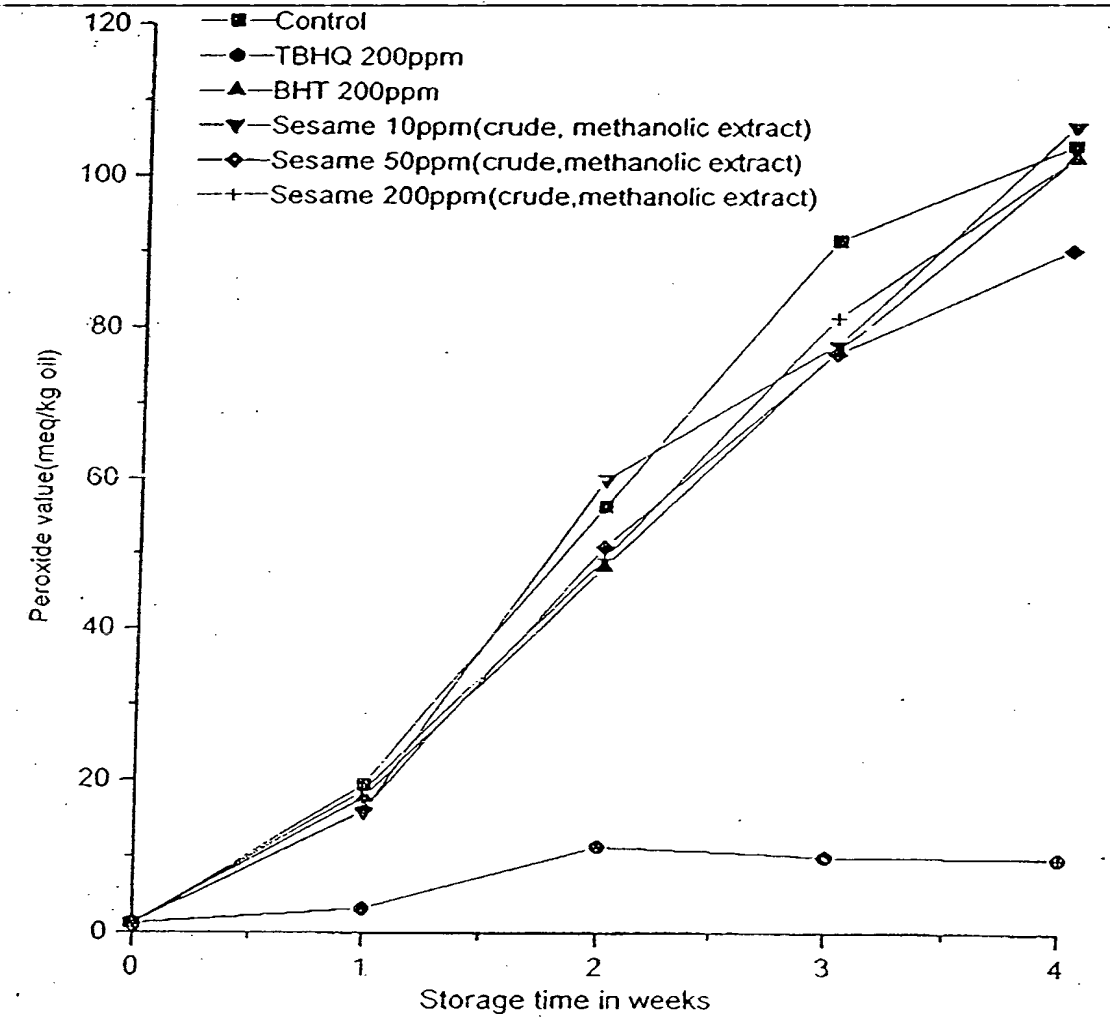

Fig.1a.Peroxide value(milliequiv. O_2/Kg) of Soybean oil stored at 60°C

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